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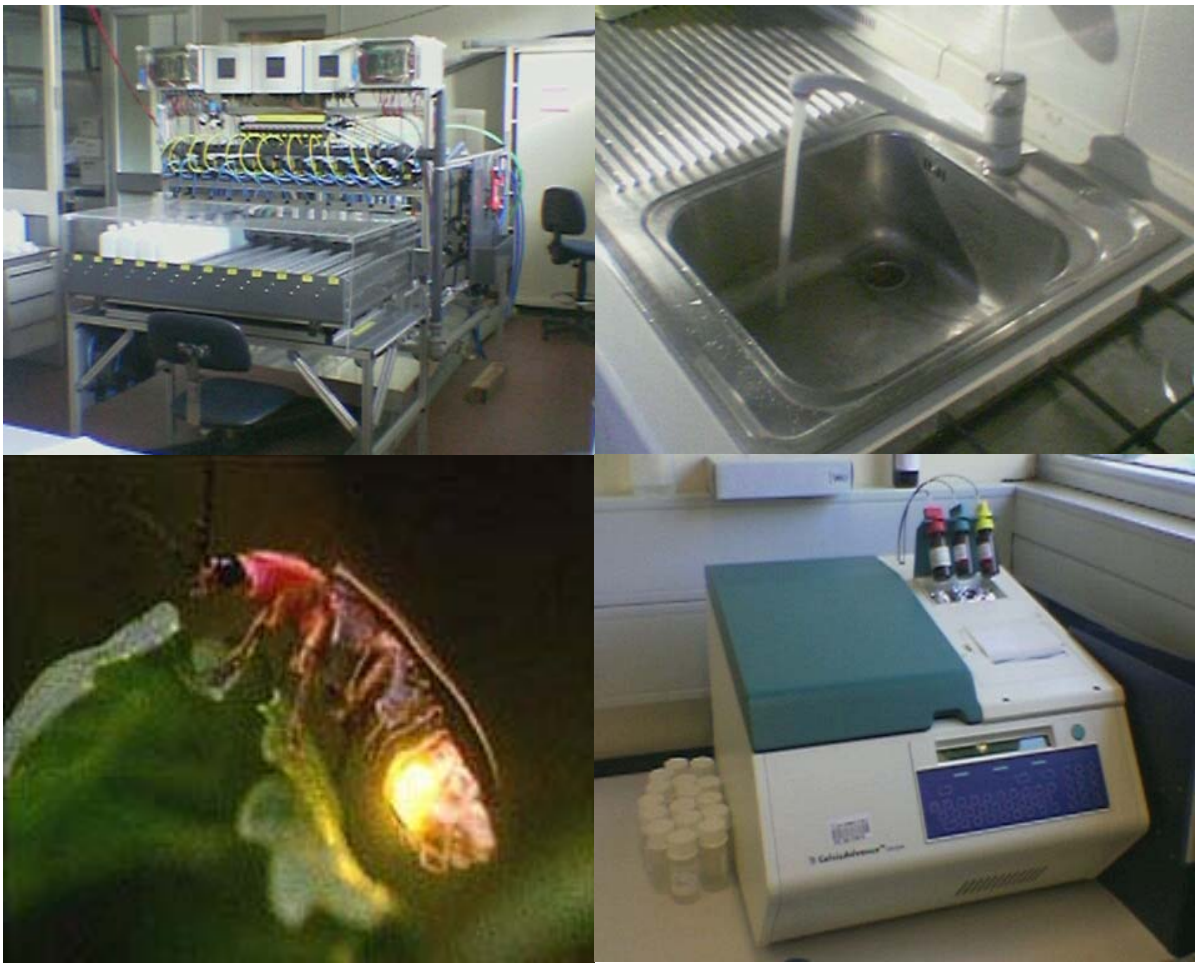

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ATP as an indicator of microbiological activity in tap water

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INTRODUCTION

The DWD [7] requires from Member States to take all measures to ensure that water intended for human consumption is free from any micro-organisms, parasites and from any substances, which in numbers or concentrations, constitute a potential danger to human health. Traditional methods to evaluate the presence of bacteria in drinking water samples, such as Heterotrophic Plate Count (HPC), need a few days of incubation and require selection of appropriate temperature and medium. Another important disadvantage is that a small fraction of microorganisms is able to cultivate on artificial mediums [1].

Among others, ATP is a general indicator for the presence of living cells. ATP can be measured in a very sensitive way, using firefly extracted from Photinus pyralis. The light emission is in the range between 500 to 700 nm wavelength [16] and the assay requires the presence of the luciferase, luciferin, magnesium and oxygen (Figure 1). The measured amount of light is proportional to the ATP in the sample. In optimum conditions 1 photon of light is produced by 1 molecule of ATP [22].

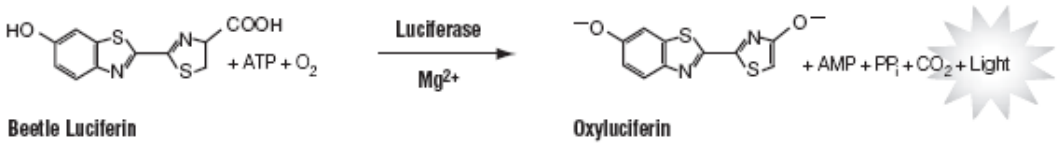

Figure 1 The luciferase reaction.

None of the other nucleotide triphosphates are active as substrates and therefore ATP can be measured in biological samples without interference from other naturally occurring compounds [10]. Adenosine triphosphate plays essential role in cell metabolism, is present in high concentrations compared with other metabolites and is uniformly distributed in the protoplasm of microorganisms from where it may be readily extracted. Due to its high rate of turnover ATP could be a good index of cell viability [13]. A number of studies have indicated that bacterial levels of ATP are correlated with cell numbers [5,8,9,12,13,14]. Wide range of bacterial ATP levels has been reported in the literature [5,8,12,13,21]. The levels will vary not only between different species of bacteria, but also within any particular species depending on the constituents of the growth medium [2,6,11] growth phase of the organism at the time of sampling [2], oxygen tension [6].

The goal of this report is to explore the use of ATP as an indicator of microbiological activity in tap water.

METHODS AND MATERIALS

Reagents

Celsis LuminATE is the light generating reagent containing luciferin and luciferase. It is supplied as a freeze-dried pellet and stored at 5° C. It is reconstituted with 7 ml of *Celsis LuminATE Buffer* and is stable for 24 h at room temperature [4].

Celsis LuminATE Buffer is used for *LuminATE* and *ATP Standard* reconstitution. It is stored at 5° C [4].

Celsis LuminEX is used for the destruction of the microbial cell membrane. It is stored at 5° C [4].

ATP Standard is supplied by Celsis in freeze-dried form. Each vial of adenosine-5'triphosphate contains 10 µg of disodium salt, 0.025 M Hepes buffer, $MgSO_4$, EDTA, sodium azide and 0.02 mg bovine serum albumin [4]. Small aliquots containing 100 µl of 2 mg/l ATP solutions after reconstitution with *LuminATE Buffer* were immediately frozen and stored at -80 ° C in capped tubes. During the performance of the analyses reagents were maintained at room temperature.

Milli-Q water was obtained from a combined Elix-Element system (Millipore). Tap water initially passes through a Progard™ pretreatment pack. It is designed to remove free particles and free chlorine from the water. The water is pressurized with a pump and than is purified by reverse osmosis (RO). Afterwards the RO product water passes through an electrodeionisation (E.D.I) module, where organic and mineral contaminants levels are reduced. Pre-treated water is exposed a 185/254 nm UV lamp to ensure the destruction of organisms, including those with trapped metals. The released elements can than be retained by the ion exchange resins. Afterwards water goes through the G-Gard polishing packs, which contains high quality ion-exchange mixed bed resin in a pure natural polypropylene housing selected for its low leaching characteristics. Final filtration is ensured through a 0.1 µm filter containing ultra high molecular weight polyethylene membrane able to remove trace ions and oxidation by-products produced by the action of the UV light [18].

Sterile water produced by Monico SPA is bought in glass bottles containing 500 ml. It is prepared by reverse osmosis followed by distillation and sterilization at 120° C.

ATP analysis

ATP was analyzed using a luminometer (Celsis Advance™ Coupe). 100 µl of the sample was put in the ATP-free disposable polystyrene tube and put in the autosampler. For the determination of free ATP, i.e. the fraction not present inside the cells, 100 µl of the mixture of luciferin-luciferase (Celsis, *LuminATE*) was injected into the sample by the automatic dispenser. For the determination of total ATP, i.e. free and cellular ATP, first 100 µl of a reagent that destroys the microbial cell membrane (Celsis, *LuminEX*) was added followed by the mixture of 100 µl of luciferin-luciferase (Celsis, *LuminATE*).

The *background measuring time* and *sample measuring time* were set at 10 s. The *background measuring time* is the measuring time prior to the actual injection of the reagents. The *sample measuring time* is the time that the instrument reads the sample tube, where the bioluminescent reaction is taking place.

To enable proper dissolution and reaction of LuminEX in the sample, the injection of LuminATE was delayed by 30 sec. A delay of 2 sec. was used between the injection of LuminATE and the measuring

time. The luminometer is calibrated to measure optimally at 560 nm wavelength of light emitted by the luminescence reaction [3].

Following technical specifications the start-up and shut-down procedure by rinsing and washing the reagents out of the tubing has been applied every measuring day. Prior to calibration and analyzing samples, blank empty tubes have been analyzed to check the background response [3].

Heterotrophic Plate Count

Water samples have been cultivated on two different mediums: R2A and PCA [19] for total counts of heterotrophic bacteria. 1 ml of the sample was placed in the Petri dish on both mediums. The plates were incubated at 22°C for 68 hours and at 37°C for 44 hours. Additionally for each medium and temperature blank mediums have been prepared.

Samples

Samples have been collected from tap water on the premises of JRC in Ispra, Italy and in surrounding villages. The volume of the samples was 50 or 500 ml. Sampling bottles before sampling have been thoroughly washed: firstly in an automatic washing machine, subsequently washed with 1% nitric acid solution and followed by three times with *Milli-Q water*.

The tap sampling procedure was consisted of flushing the tap for one minute and taking directly a sample first followed by a known stagnation period and sampling two or more successive samples.

Additionally samples have been obtained using the Dynamic Test Facility. The *Dynamic Test Facility* is a device that simulates consumer behaviour enabling to measure automatically temperature, pH, conductivity and dissolved oxygen of the incoming water. The facility is used to study corrosion and the potential of materials to form a biofilm. Currently 4 lines with different pipe materials are in use: copper, stainless steel, galvanized, polypropylene. The DTF has been programmed to take samples automatically after stagnation times of 0.5, 1, 2, 4, 8 and 16 h. Since 0.5 and 1 h stagnation (0.5 HS and 1 HS) occur twice in the measuring procedure, the nomenclature 1st and 2nd stagnation have been used. The volume of all DTF samples is 200 ml.

CALIBRATION CURVE

On each measuring day a new ATP stock solution containing 100 µl of 2 mg/l ATP has been used. After thawing for half an hour at a room temperature, the stock solution was 1000-fold diluted followed by steps of 10-fold dilutions down to 2 ng ATP/l. This latter solution was diluted 2-fold (1 ng ATP/l). On each day, a calibration curve (Figure 2) for total and free ATP concentration has been prepared. Annex 2 gives the details for all curves.

As a solvent for ATP dilutions firstly *Milli-Q water* and *sterile water* have been used. However, considering the fact that this may lead to underestimation of ATP concentrations in samples, due to the composition of the tap water [22] further dilutions have been prepared using water from water production point in JRC-Ispra, Italy.

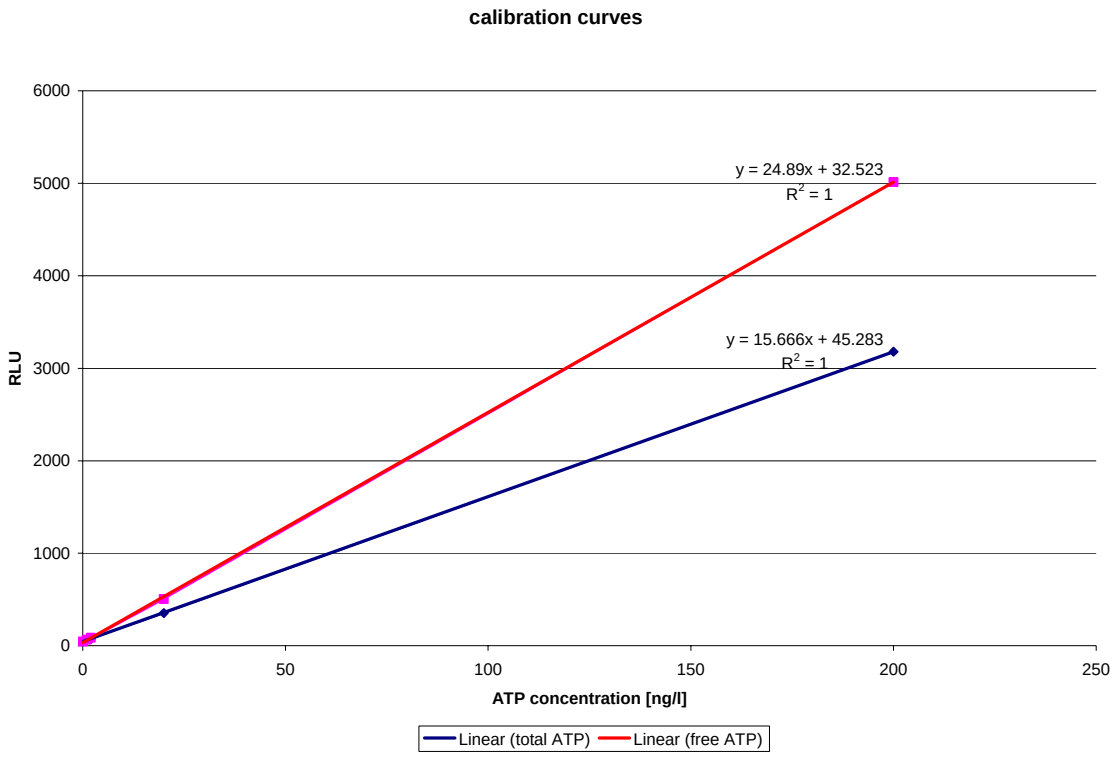

Figure 2 Calibration curves for total and free ATP

Slopes obtained through linear regression of all calibration curves (prepared on each measuring day) are illustrated in Figure 3 for total ATP and Figure 4 for free ATP. The average slope of calibration curve for total ATP is 15±2.29 and for free ATP 25±2.60.

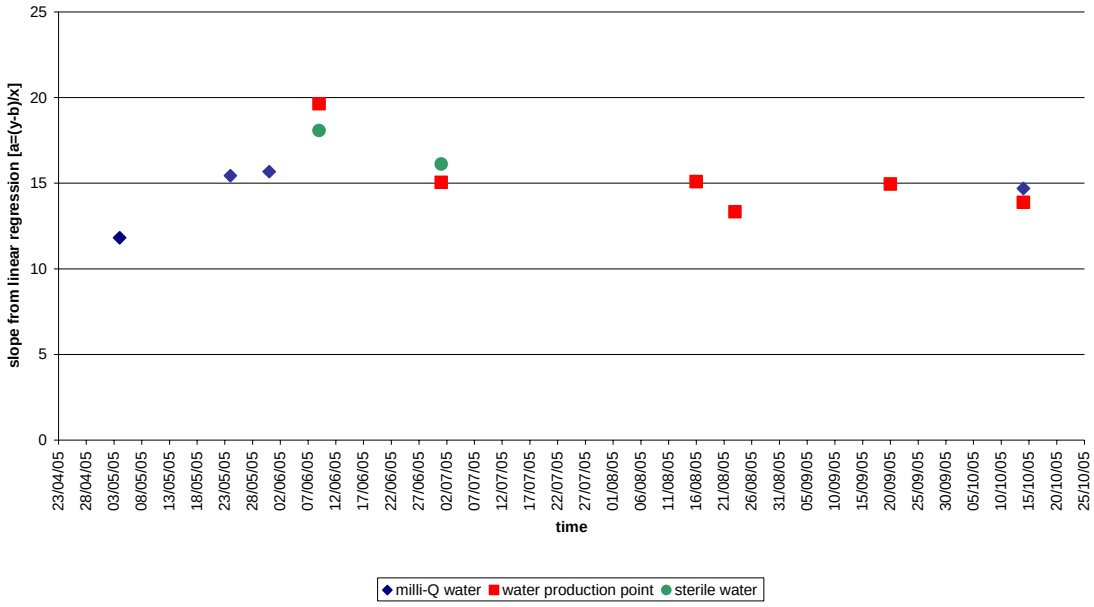

Figure 3 Slopes of calibration curves for total ATP obtained with different water types

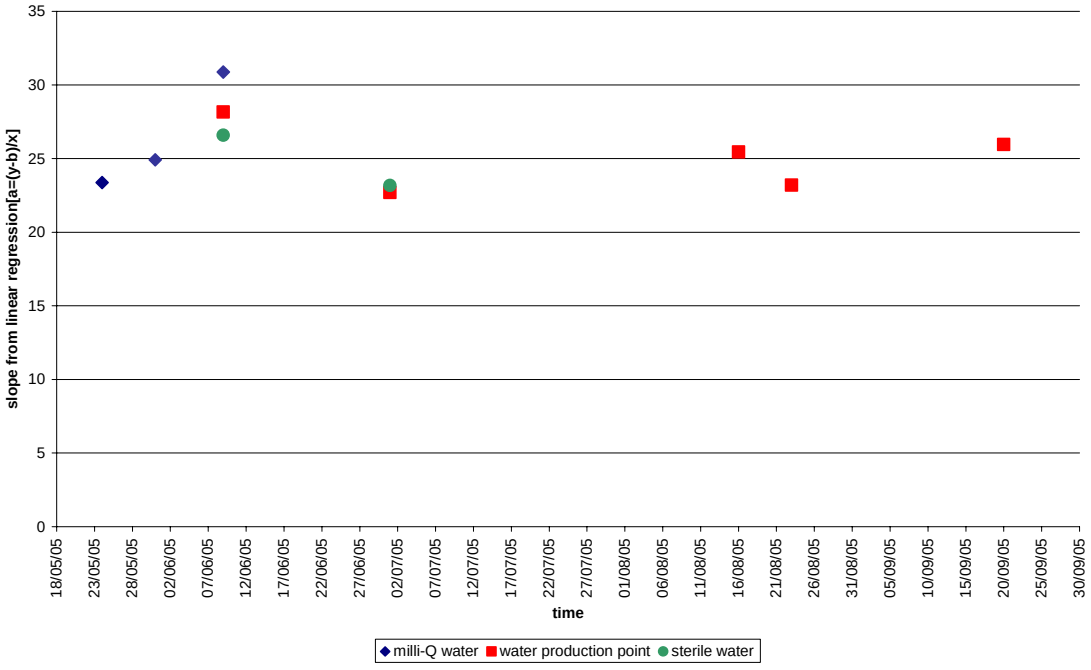

Figure 4 Slopes of calibration curves for free ATP obtained with different water types

DETECTION LIMIT OF ATP

To estimate the limit of detection that is significantly different from the background, the comparison of the RLU average of two concentrations was performed. It was assumed that the standard deviations of the two means are not significantly different [17]:

Eq.1 $\mathbf{S^2 = ((n_1-1)s_1^2+(n_2-1)\ s_2^2)/(\ n_1+\ n_2-2)}$

n_1 amount of water samples (milli-Q water samples)
n_2 amount of samples with 0.2 ng/l ATP concentration
s_1 standard deviation of water samples (milli-Q water samples)
s_2 standard deviation of samples with 0.2 ng/l ATP concentration

Eq.2 $\mathbf{t = (x_1-x_2)/s/(1/n_1+1/n_2)^2}$

x_1 mean of water samples (milli-Q water samples)
x_2 mean of samples with 0.2 ng/l ATP concentration
t has $\mathbf{f = (n_1+\ n_2)-2}$ **degrees of freedom**

Annex 1 gives the data of milli-Q water, 0.2, 1, 2, 20, 200 ng/l of ATP. As an example the hypothesis was tested that the two RLU means obtained for Milli-Q water samples and 0.2 ng/l ATP samples are equal. There are 10 degrees of freedom for comparison of Milli-Q water samples and 0.2 ng/l ATP samples. The calculated |t| value is 1.42. The critical value of |t| (P=0.05) is 2.23. Since the critical |t| (P=0.05) value is higher than the calculated value, the difference between RLU values of Milli-Q water samples and 0.2 ng/l ATP samples is not significant.

Using the same statistical test RLU values of the following averages have been compared:

a. Milli-Q water and 1ng/l ATP

b. 0.2 ng/l ATP and 1 ng/l ATP

c. 1 ng/l ATP and 2 ng/l ATP

The test show that the critical values of |t| (P=0.05) for the above samples are lower than the calculated |t| values, therefore the differences between RLU means for these cases are significant. The concentration of 1 ng/l ATP is a good estimate of the limit of detection since it is significantly different from milli-Q water and 0.2 ng/l ATP.

CALCULATION OF ATP CONCENTRATION IN THE SAMPLES

The method for calculating the concentration of ATP in a sample has been two times modified due to the fact that initial calculations gave negative values of ATP concentration in the samples (Eq. 3 and Eq. 4). It happened especially when "b" value in Eq. 3 was very high, what suggested that water used for ATP dilutions was not ATP free. In the next equation this situation occurred when RLU value of the sample was lower than background value of the sample, i.e. the value measured just before adding the reagents. Considering above problems a third modification has been adopted (Eq. 5). In new method "b" values and background values of particular samples have been replaced by the mean value of the RLU values measured for empty tubes without reagents.

The average of RLU values measured for empty tubes as well as the average of background response measured for empty tubes almost overlap. Both means were obtained for the same amount of samples (60 samples). However, RLU values of empty tubes were much more homogeneous and therefore the standard deviation is smaller, i.e. 37±4 RLU (See Fig.5), in comparison to the standard deviation of the background response for empty tubes, i.e. 38±9 RLU.

Both, average of background responses of all samples as well as average of background responses of samples excluding blanks presents a value of 42.75±12.39 and 43.07±12.51, respectively. Stdev is quite high, what could be due to the fact that amount of samples is very big (876-936 samples). See Table 1.

All data and calculations are presented in Annex 4.

Eq.3 $X = (y-b)/a$

Eq.4 $X = (y-background)/a$

Eq.5 $X = (y- factor)/a$

where:

y	RLU value of a particular sample
b	constant value received from the linear regression of the calibration curve (y = ax+b)
background	background value as measured for a particular sample just before adding the reagents
a	coefficient corresponding to each x-value received from the linear regression of a calibration curve (y = ax+b)
factor	mean value of RLU values measured for empty tubes without reagents. The value of the factor is 40 RLU.

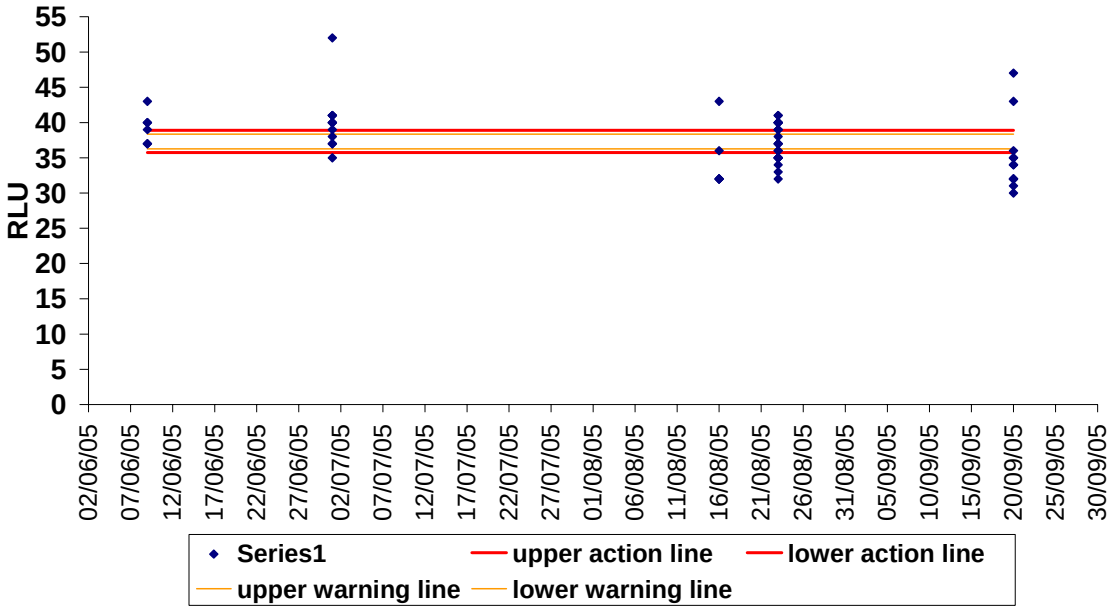

Figure 5 Shewart chart for RLU values of empty tubes

Table 1 Averages and standard deviations of background and RLU values in the samples and blanks

Samples	mean	stdev	amount of samples
RLU values of empty tubes	37.31	4.08	60
background values of empty tubes	38.16	9.47	60
background values of all samples	42.75	12.39	936
background values of samples excluding blanks	43.07	12.51	876

RLU RESPONSE

The assay was performed on samples containing only milli-Q water in order to check how different variable would modify the response. Different volume of milli-Q water samples seems not to have a significant influence. RLU values were fluctuating in the range 35-45 RLU. Sunlight exposure was another parameter of interest. Although some samples gave elevated RLU values after sunlight exposure has been applied others didn't change the response. Additionally, since all tubes used in experiments were polystyrene, an "antistatic test", to see if material of tubes have any influence on the response, have been included.

Effect of sample volume

An experiment has been conducted to check if volume of the sample has an influence on the response Samples with different volumes of Milli-Q water have been prepared and measured immediately without adding reagents.

RLU values between 6 samples with 100 µl milli-Q water, 6 samples with 200 µl milli-Q water and 6 samples with 300 µl milli-Q water don't differ significantly. The conclusion is that the volume of water sample is not important. However, repetition of samples containing 100 µl milli-Q water showed higher RLU values (see Fig.6 and Annex 3).

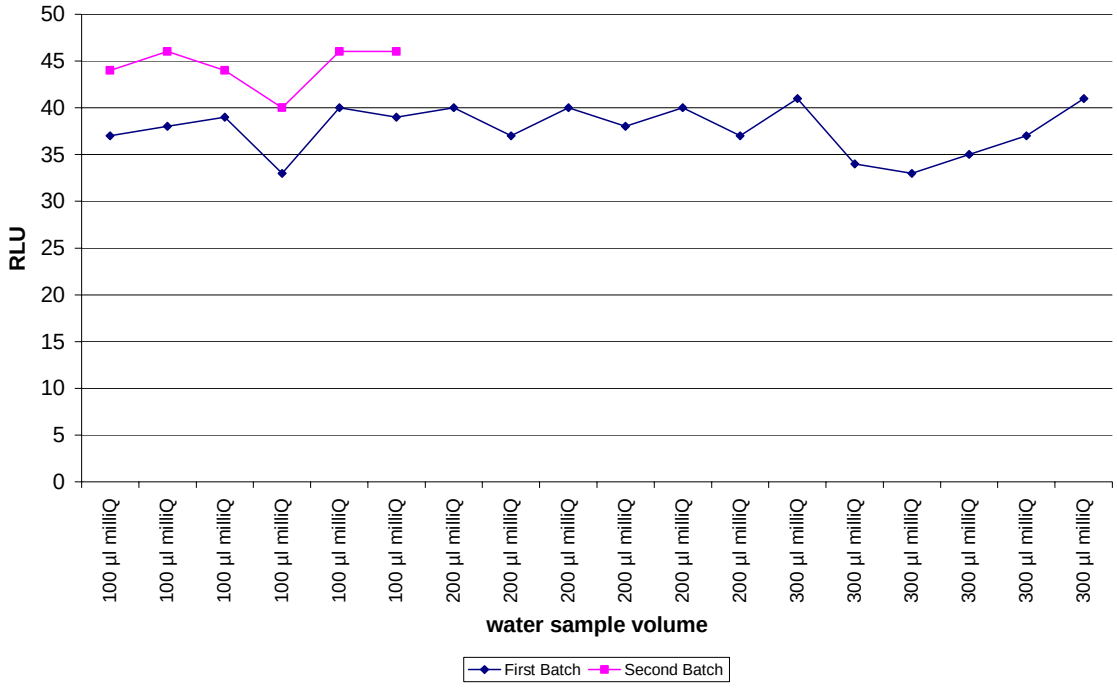

Figure 6 Influence of volume in water samples on RLU values

Effect of ambient light

Purpose of this experiment was to see if samples exposed to sunlight will give different response from samples that were not exposed. Tubes have been filled with 100 µl of Milli-Q water and exposed to sunlight for different periods of time.

RLU values between samples with water exposed on sunlight vary between 40-80 RLU. One can notice that after 2 min on sunlight exposure a significant increase in RLU values can be observed. RLU values of 2 min, 5 min and 10 min samples are very similar and significantly different from 0 min samples. Subsequently samples kept for 2 minutes on sunlight and afterwards during 2 minutes in the dark chamber of the luminometer prior to measurement, show a significant reduction in RLU values. After 40 min, 80 min and 160 min of sunlight exposure even higher RLU values can be observed (significantly different from 0 min. samples). However samples exposed through 20 min and 320 min show lower RLU values. 20 min samples and 0 min samples don't show any significant difference in contrast to 320 min samples that are significantly different from 0 min samples. Results are presented on Fig.7 and in Annex 3.

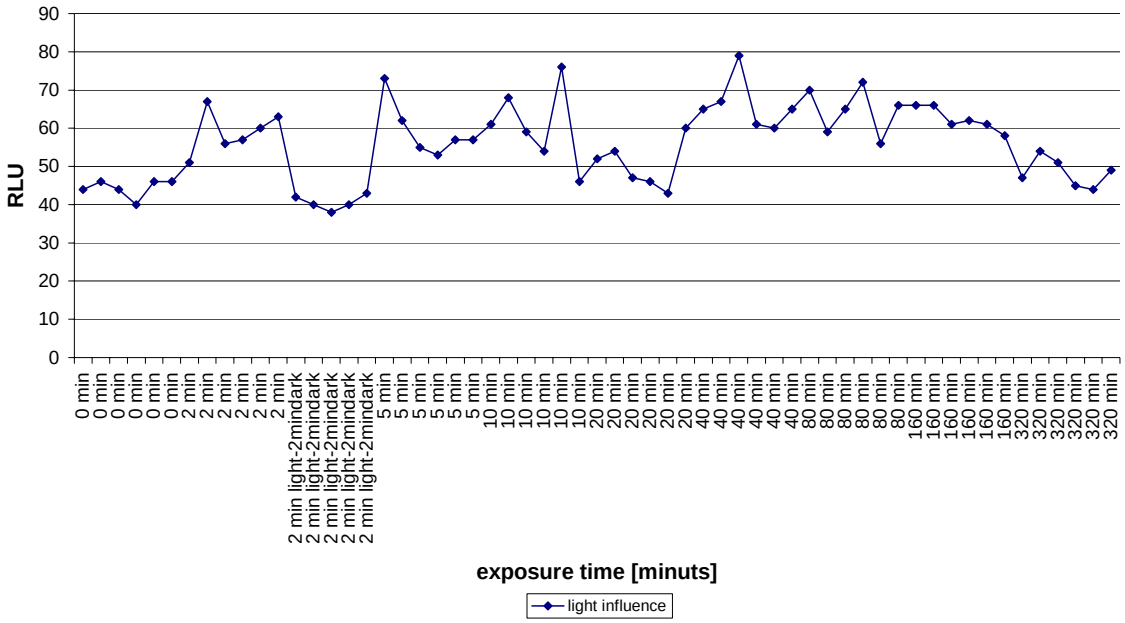

Figure 7 Sunlight exposure of water samples.

Experiment on sunlight exposure has been repeated after few days. Samples have been exposed on sunlight but additionally tubes prior to measuring were made antistatic with the ethanol. All samples had the same volume 100 µl of Milli-Q water. Samples in the last set have been rubbed with a synthetic blouse to check if polyethylene tubes containing water samples would change RLU values being initially antistatic. For better comparison results of this experiment as well as the previous one are presented together in Fig. 8 (see also Annex 3).

RLU values of 0 min-ethanol samples are significantly higher comparing to those obtained in previous experiment 0 min samples. On the contrary RLU values of sunlight exposed 2 min-ethanol samples are rather low comparing to previous results (2 min). However RLU values of 2 min light-2 min dark-ethanol samples and 2 min light-5 min dark-ethanol samples are comparable to RLU values of 2min light-2 min dark samples from the previous experiment. RLU values of these samples are similar to non-exposed samples (0 min). Tubes charged with static electricity by rubbing with the synthetic blouse and measured just after the preparation show low RLU values. This suggests that the luminometer is able to discharge the static electricity and that there is no need to make the tubes

antistatic. However, the control of analyzing empty tubes serves to control if the luminometer as a whole is not charged and thus influencing the measurements.

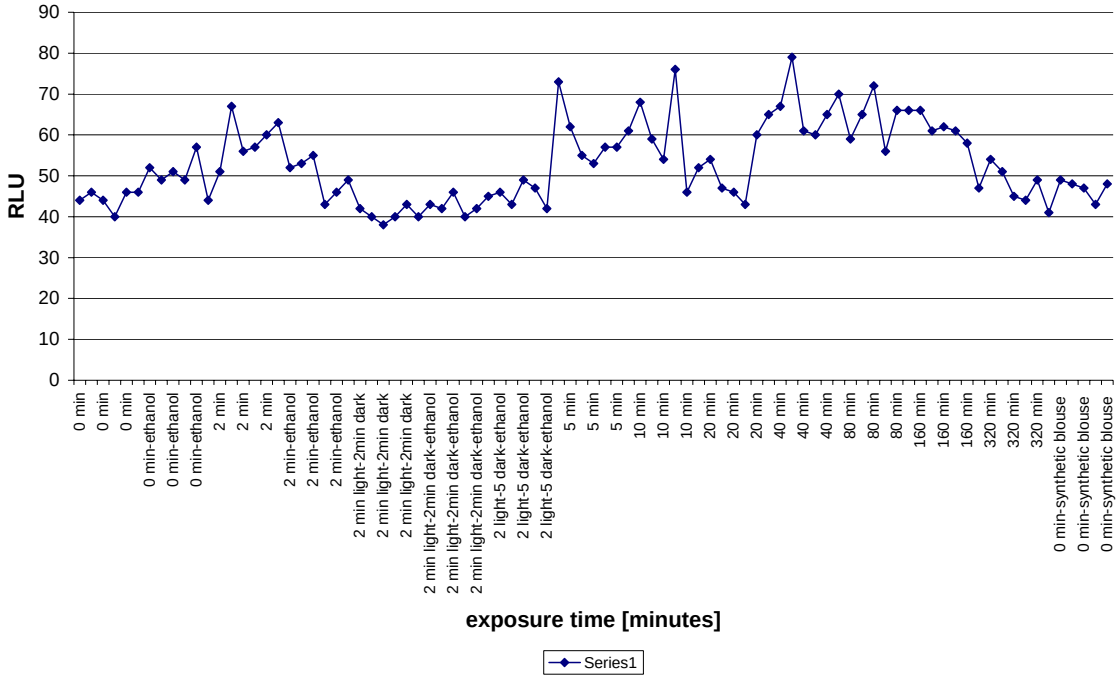

Figure 8 Sunlight exposure and antistatic test of water samples

Effect of LuminEX on response

Samples with the same ATP concentrations have been measured for total ATP and for free ATP. The purpose of the experiment was to verify the influence of *LuminEX* on the RLU values.

Samples with 100 µl of 200 ng ATP/l have been separated for two batches. In one batch 100 µl of Milli-Q water and 100 µl of *LuminATE* has been added, while in the second batch 100 µl of *LuminEX* and 100 µl of *LuminATE* have been added. The overall volume of samples in both batches was therefore 300 µl. ATP concentrations have been prepared accordingly to *Calibration curve procedure,* as a solvent for ATP Standards Milli-Q water have been used.

The average RLU value of the samples without LuminEX addition (3125.67±105.3) compared to average value of the samples with addition of LuminEX (2774.33± 155.5) is significantly different. The probable explanation is that *LuminEX* is absorbing the light that is produced in the luciferin-luciferase reaction. Consequently RLU values obtained for samples where both reagents were added are lower than those where only *LuminATE* was added (See Annex 3).

Effect of LuminATE volume on response

The purpose of this experiment was to check if the RLU value obtained from the samples with the same ATP concentration (200 ng/l ATP) is incident to the concentration of *LuminATE*. Therefore samples have been made with variable volumes of *LuminATE* and Milli-Q water, while keeping the overall volume constant, thus 300µl.

The measurement shows that the volume of *LuminATE* has influence on the RLU values. The higher the concentration of *LuminATE* the higher is the response. Results are presented on Fig.9 and in Annex 3.

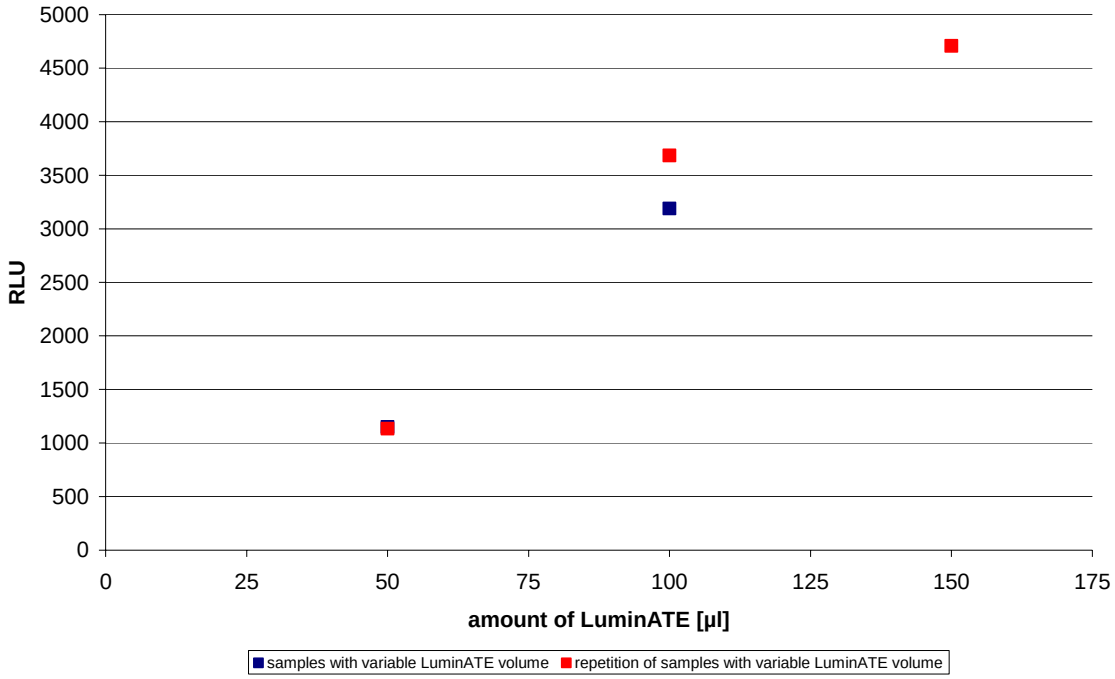

Figure 9 Influence of LuminATE on the RLU values

ATP STABILITY AND DEGRADATION

To assess ATP stability and degradation the following experiment has been conducted. 100µl of 2mg/l ATP solutions have been diluted in water from the water production point and in Milli-Q water to see if degradation depends on the type of water. All dilutions were prepared in successive dilution using first a 1000 time step followed by 10 times dilution down to 2 ng/l. The latter was diluted to 1 ng/l.

Also blank water samples (without ATP addition) of water from the water production point and milli-Q water were included in the experiment. Due to fact that it was difficult to take the whole ATP aliquot from the vial to prepare dilutions, real ATP concentrations in samples prepared on different days could vary significantly giving diverse RLU values.

Samples have been kept for 4 days in 4^{o}C and 24^{o} C environment. All the samples during storage have been capped. On the measuring day 20th of September also new ATP dilutions for calibration curve have been prepared and directly afterwards analyzed (see Annex 2).

In order to have more data and trace more in detail ATP degradation, the experiment on ATP degradation has been modified and repeated. ATP dilutions have been prepared in the same way as previously but stagnation time has been changed. Dilutions of ATP Standards have been prepared and stored in 4^{o}C and 24^{o}C environment for 1 day, 4 days, 8 days, 9 days and 10 days. As previously, on the day of analysis also new ATP dilutions for calibration curve have been prepared (see Annex 2).

Calculation of ATP concentration in samples prepared for ATP degradation

ATP concentrations in the samples prepared for ATP degradation have been firstly calculated using X=(y-factor)/a method. However calculated in this way ATP concentrations especially in samples with low initial ATP concentrations (1 ng/l ATP and 2 ng/l ATP) gave always too elevated calculated ATP concentrations in proportion to initial ATP concentrations in those samples. That could be due to the fact that RLU values of blank tap water samples were very similar to RLU values received from samples containing 1 ng/l ATP and 2 ng/l ATP. Therefore the responses of the samples have been corrected by the RLU response of the blank before the calculation method was applied. This new method has also given negative concentrations: e.g.: all ATP samples prepared with tap water after 10 days of stagnation in 24^{o} environment gave lower RLU values than blank tap water sample also stored for 10 days at 24^{o} (See Annex 5).

It appears that ATP stability and degradation depends on the type of water used for the dilutions. ATP is rather stable when diluted in *Milli-Q water* regardless the temperature and time. On the other hand ATP diluted in tap water show gradual degradation with time especially at 24^{o} C. 4-5 days seem to be a half-life of ATP especially for 200 ng/l ATP and 20 ng/l ATP concentrations. Samples with lower concentrations: 2 ng/l ATP and 1 ng/l ATP show slower ATP degradation and their half-life can be estimated as 8-9 days. After 10 days only trace amounts of ATP remained in the samples (Fig.10).

ATP concentrations prepared with tap water and kept in 4^{o}C environment show much slower ATP degradation comparing to samples prepared with the same water and kept in 24^{o}C environment. Half-life for all ATP concentrations in this temperature can be assessed as 8-10 days (Fig. 11).

ATP concentrations prepared with milli-Q water and kept at 4 ^{o}C and 24^{o}C seem rather stable with time (Fig.12 and Fig.13). Unexpectedly the initial concentration of 200 ng/l seems to increase with time. However it might be explained by the precision of the analysis and the way the samples have been prepared.

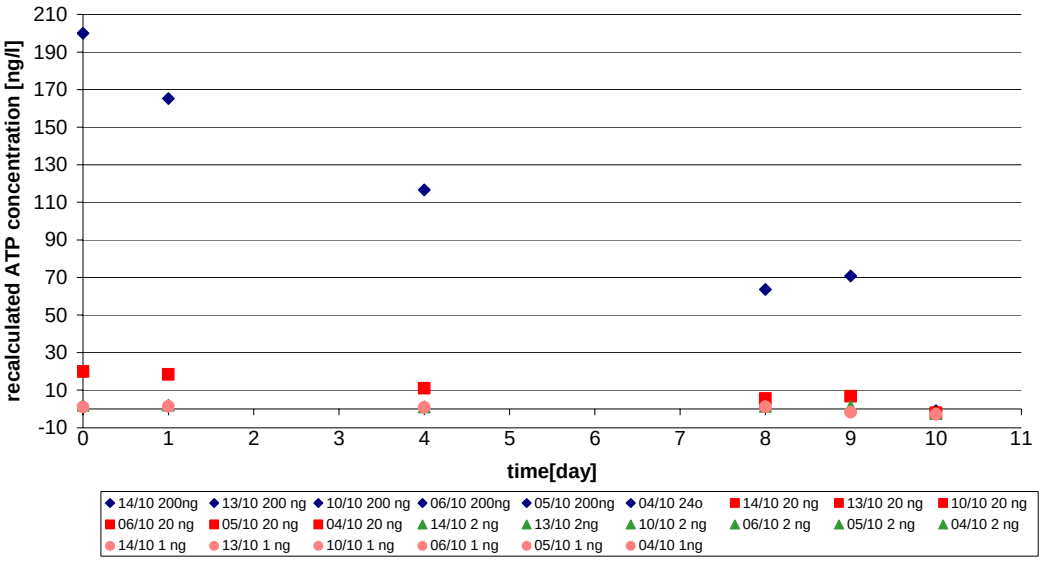

Figure 10 Tap water samples stored in 24°C

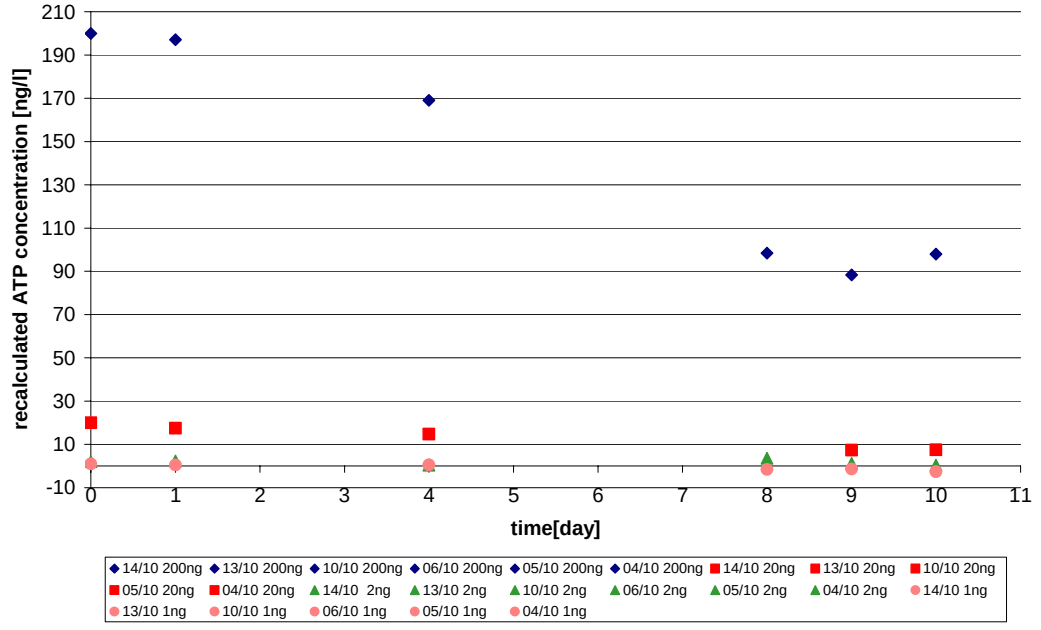

Figure 11 Tap water samples stored in 4°C

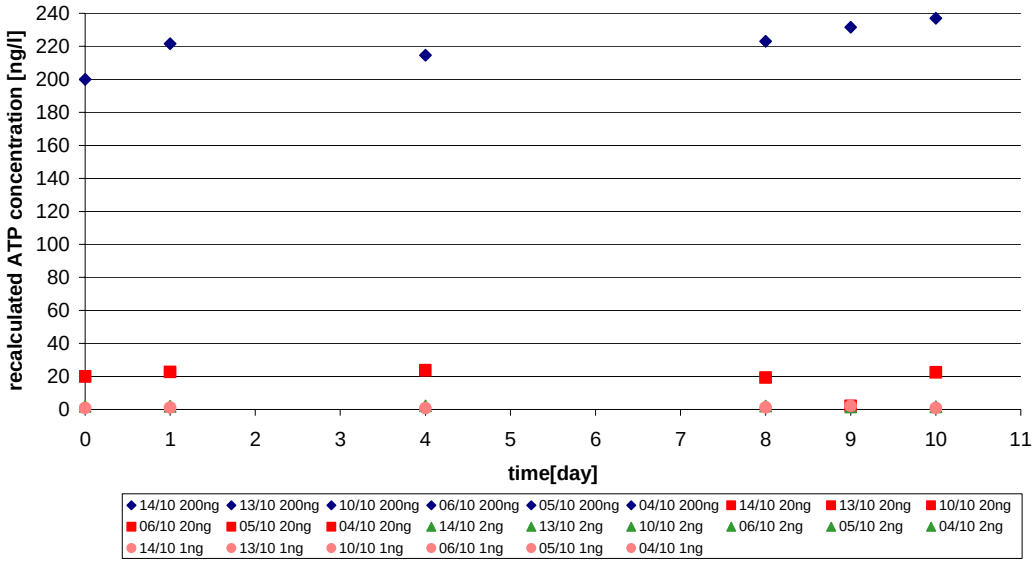

Figure 12 Milli-Q water samples stored in 24°C

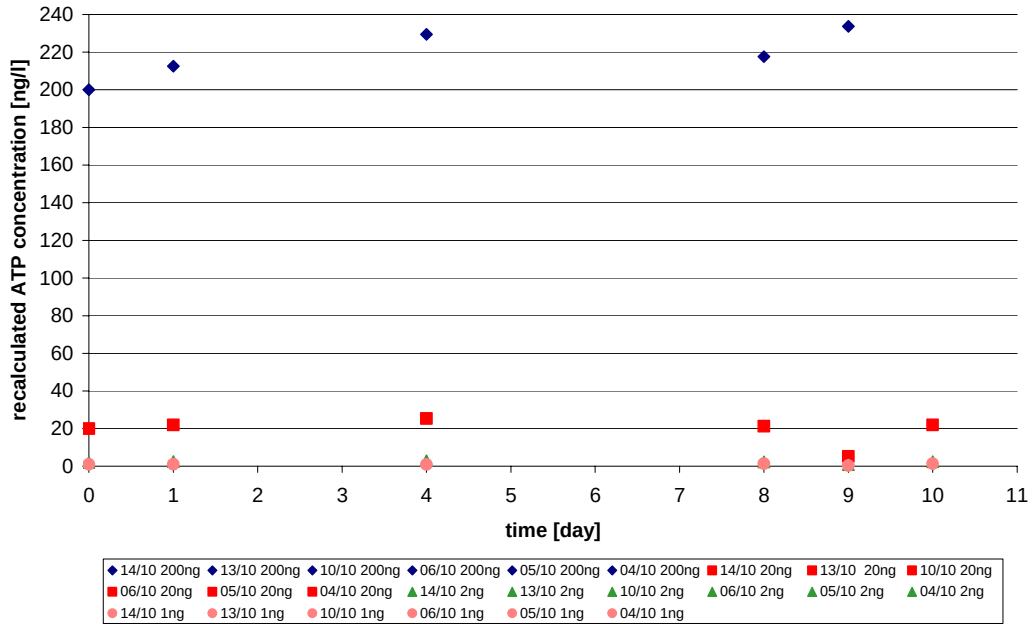

Figure 13 Milli-Q water samples stored in 4°C

INFLUENCE OF STAGNATION TIME ON ATP CONCENTRATION IN TAP WATER SAMPLES

500 ml samples at consumers' tap in Leggiuno and Laveno

The aim of the experiment was to scrutinize if stagnation time has an influence on ATP concentrations at the consumers' tap. 15 samples with defined stagnation times have been taken from two private houses in Laveno and in Leggiuno and measured for total and free ATP. The sample procedure was as follows. The tap in the kitchen was flushed with the tap fully open for 1 min. A FF sample of 500 ml was taken and the tap was closed for stagnation. After 0.5 h (about 9.30-10.00), 1 h (about 10.00-11.00), 2 h (about 11.00-13.00), 4 h (about 13.45-17.45) and 8 h (about 23.00-7.00) stagnation, two successive samples of 500 ml were taken. Experiment has been conducted on 23/08/05 and 20/09/05 (see Annex 6.1).

Fig. 14 and Fig. 15 present ATP concentrations in tap water samples from Leggiuno. Samples contain concentrations of total ATP in the range of 15-52 ng/l. FF samples and short stagnation time samples of 0.5, 1 and 2 h, contain the highest ATP concentrations. With longer stagnation times of 4 and 8 h the concentration gradually decreases. Free ATP is mostly below 10 ng/l ATP, what suggests that microbial ATP constitutes the major part of total ATP.

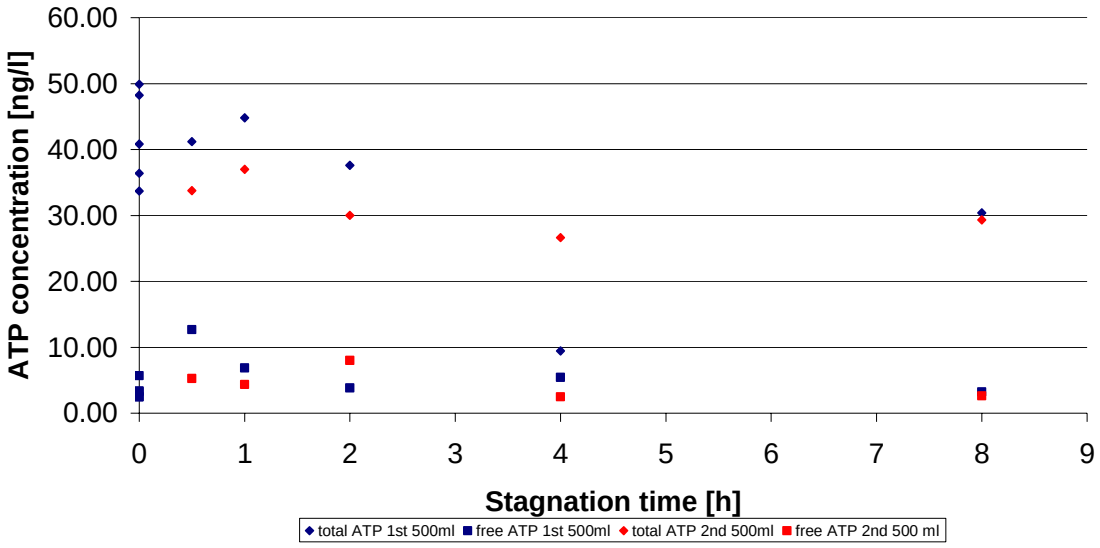

Figure 14 Dependence of ATP concentration on the stagnation time in tap water samples from Leggiuno (23/08/05)

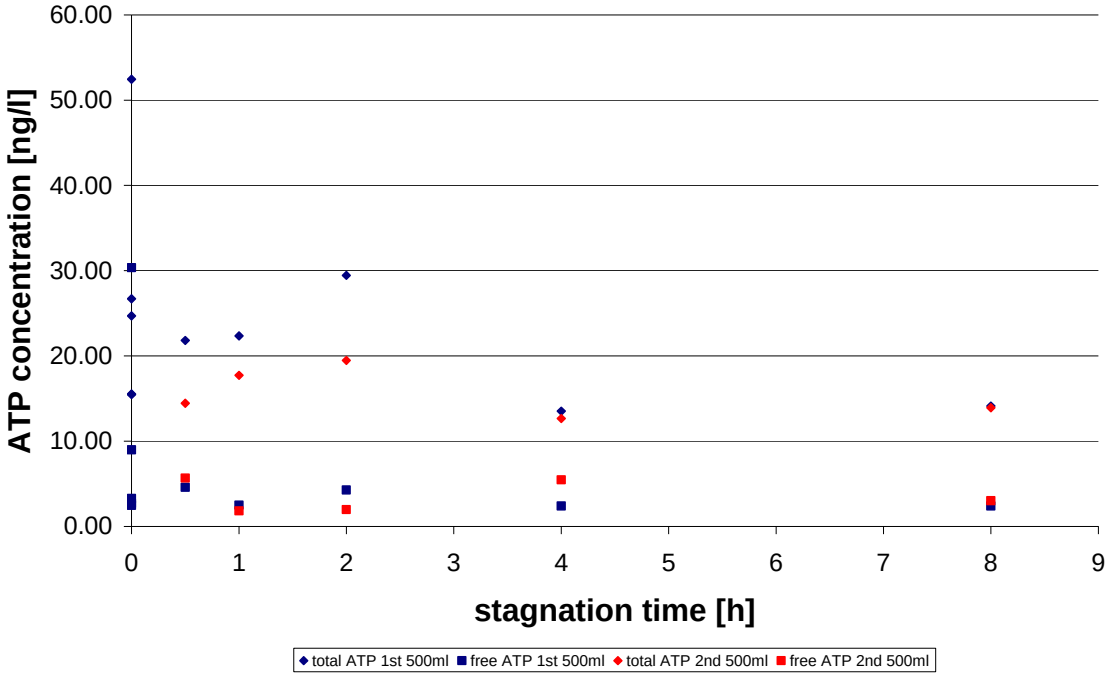

Figure 15 Dependence of ATP concentration on the stagnation time in tap water samples from Leggiuno (20/09/05)

Tap water samples from Laveno show ATP concentrations in the range of 0.5-9 ng/l ATP. In contrast to the samples from Leggiuno ATP concentrations in samples from Laveno show a completely reverse distribution. ATP concentrations increase very slowly with time (especially in the 1st 500 ml total samples). Free ATP concentrations are below 2 ng/l ATP. See Fig. 16 and Fig. 17.

It can be concluded that regardless the sampling place almost all 1st 500 ml samples (of 2 successive samples with defined stagnation time) contain higher ATP concentrations (except 8HS total samples from Leggiuno). One can also notice that the disparity between 1st and 2nd 500 ml free ATP samples is much lower comparing to the disparity between 1st and 2nd 500 ml total ATP samples.

The overall deduction is also that ATP concentrations of 23rd August samples are higher than those from 20th September. The effect of temperature may play a role here.

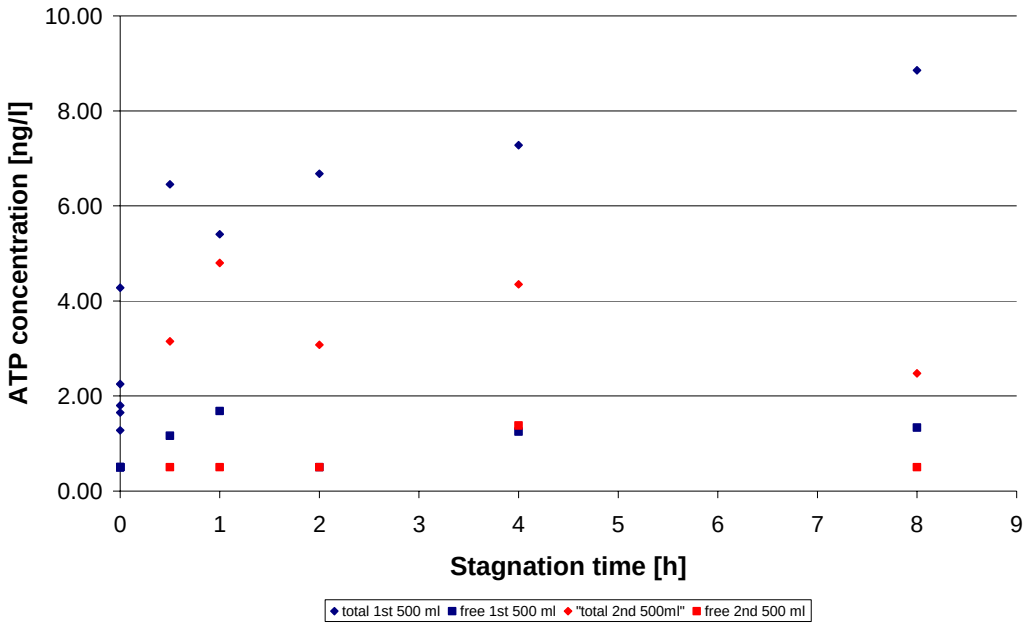

Figure 16 Dependence of ATP concentration on the stagnation time in tap water samples from Laveno (23/08/05)

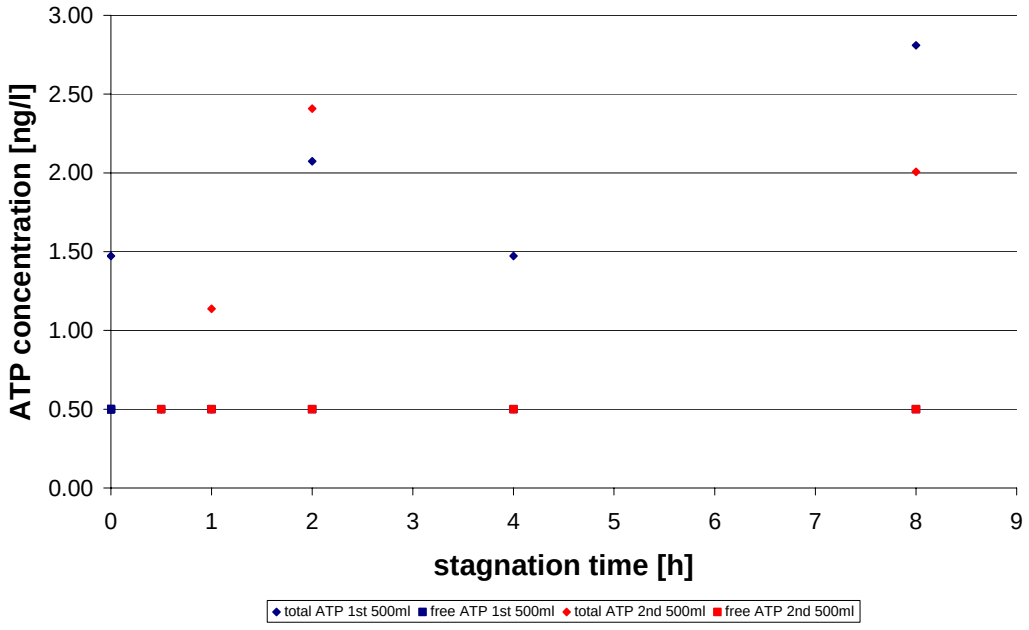

Figure 17 Dependence of ATP concentration on the stagnation time in tap water samples from Laveno (20/09/05)

ATP concentrations in 20 successive 50 ml tap water samples from Leggiuno and Laveno

In order to trace in detail the distribution of total and free ATP concentration in 1 litre volume, 20 successive 50 ml tap water samples from Laveno and Leggiuno have been taken (see Annex 6. 2). Prior to stagnation of 8 h and sampling a fully flushed sample has been taken. Samples have been gathered on 16/08/05.

Samples from Laveno show much higher total ATP concentrations in the first 10 successive samples, what confirms our observation from the previous experiment where ATP concentration in the 1st 500ml total samples after 8HS was much higher. Three first samples and 9th sample from Laveno present a little elevated ATP concentration (12-21 ng/l ATP). Other samples including FF sample are below 10 ng/l ATP concentration (Fig.18*).* The free ATP concentration doesn't change much in the samples, regardless the increase of total ATP in some samples.

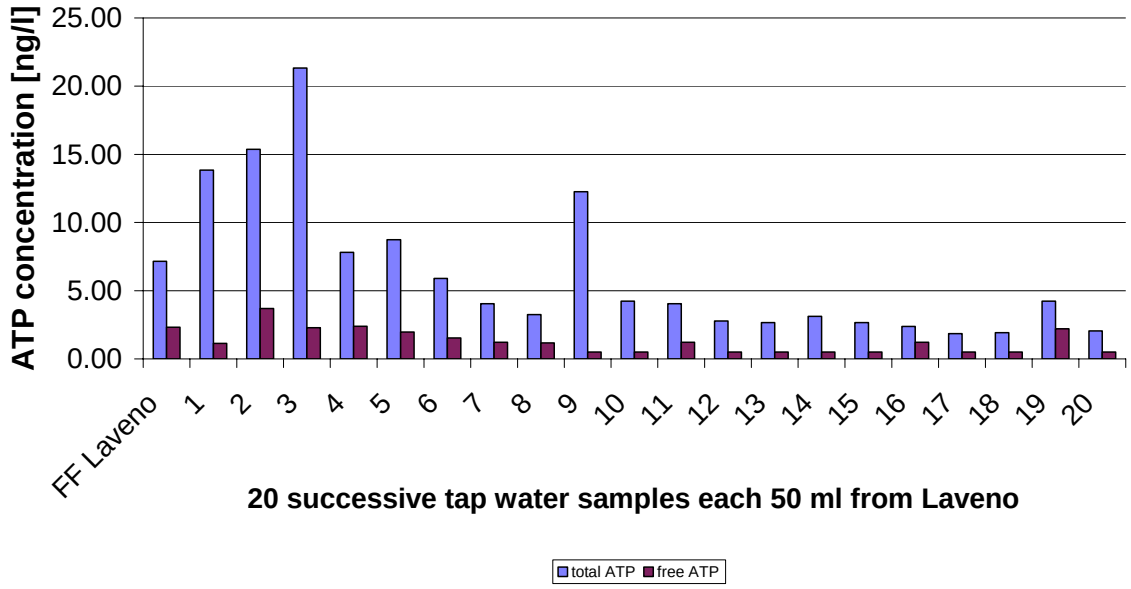

Figure 18 ATP concentration in 20 successive tap water samples from Laveno after 8 HS (16/08/05)

Samples collected in the same way in Leggiuno (Fig.19) present a completely different distribution of total ATP concentration. There isn't much difference between the samples (15-20 ng/l), although significant lower ATP concentration in the first two samples can be observed (10 ng/l). These results confirm the results from the 500 ml sampling, where the 1st 500ml total samples after 8HS had a similar ATP concentration as the 2nd 500ml sample. Free ATP concentration constitutes around 30% of total one and don't exceed 6 ng/l ATP.

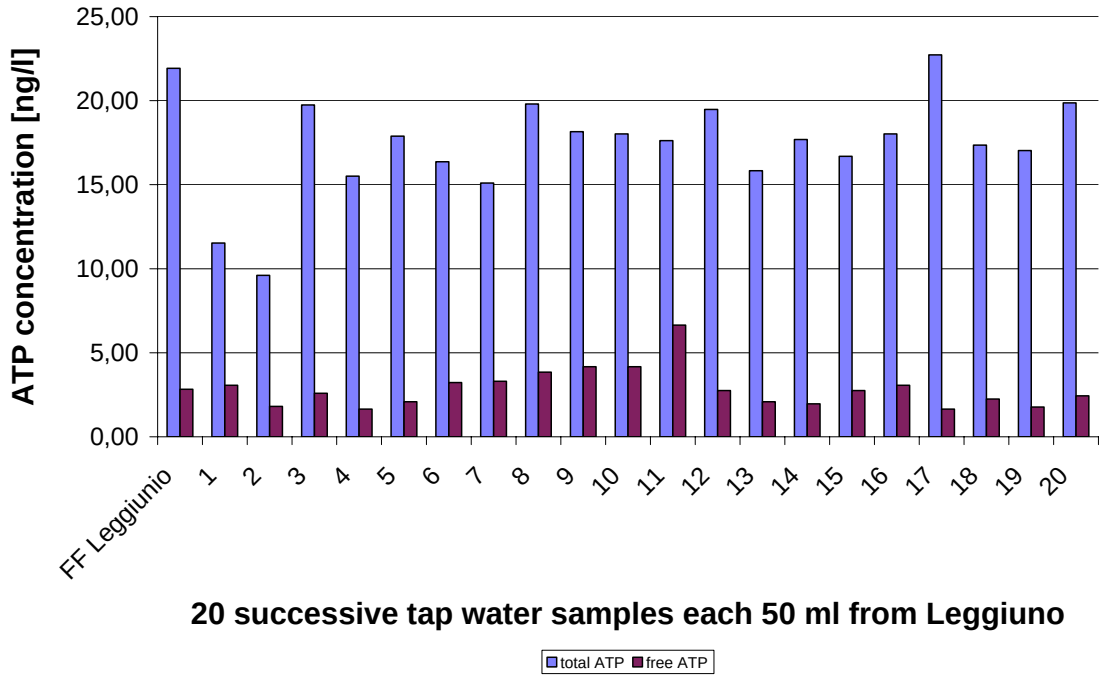

Figure 19 ATP concentration in 20 successive tap water samples from Leggiuno after 8 HS (16/08/05)

ATP concentrations in samples from the Dynamic Test Facility

The Dynamic Test Facility is programmable and simulates the water consumption by consumers. The instrument uses tap water provided by water production point (JRC, Ispra, Italy). Water before distribution is chlorinated by the addition of chlorine dioxide. Four different pipes are installed in the DTF: copper, stainless steel, galvanized and polypropylene. The DTF has been programmed to take samples after defined stagnation times, as described in experimental part. The aim of the experiment was to trace the distribution of total and free ATP concentrations depending on stagnation time in four different lines. The same experiment has been conducted on 01/07/05 and 20/09/05 (see Annex 6.3).

It can be noticed on the charts presented below that in general the ATP concentration gradually decreases with longer stagnation time for all materials. There is one exception for the galvanized pipe, where a gradual increase of the total ATP concentration is observed in samples obtained on 20th of September 2005. In addition, the ATP concentration in the first stagnation is mostly higher than in the second stagnation of the same period on the same day, with 2 exceptions for copper and polypropene on 20/09/05.

Generally ATP concentrations do not only differ between materials, but also within the same line one can notice variations in samples taken on two different measuring days.

On the 1st of July the ATP concentration was gradually decreasing in the copper pipe. On the other hand on the 20th of September 2005 an unusual distribution of total and free ATP concentration has been observed. All the samples from this day have a higher free ATP concentration then a total ATP concentration. The explanation to this phenomenon has not been found so far (see Fig.20 and Fig.21).

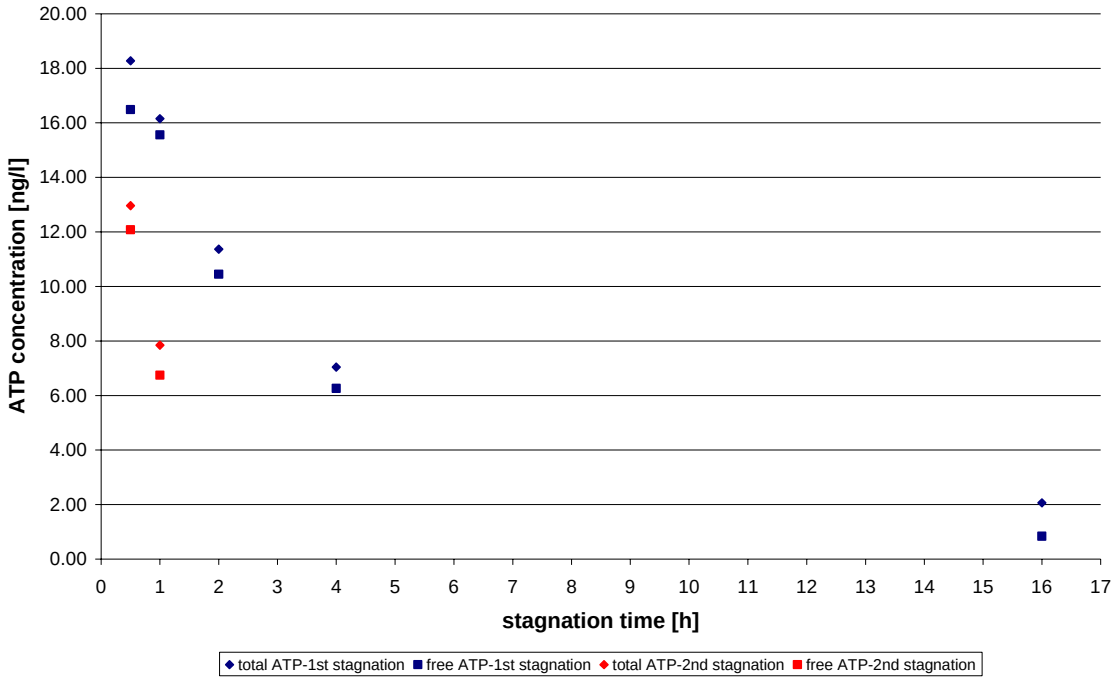

Figure 20 Dependence of ATP concentration on the stagnation time in copper pipe (01/07/05)

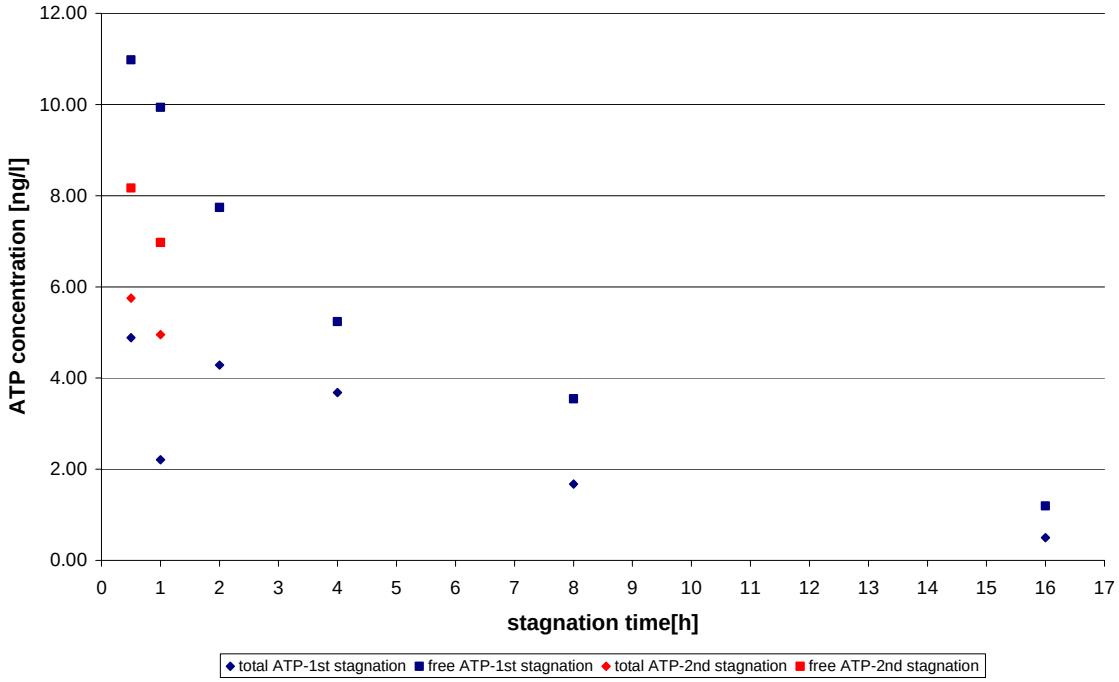

Figure 21 Dependence of ATP concentration on the stagnation time in copper pipe (20/09/05)

In the stainless steel pipe the ATP concentrations were quite elevated, up to 23 ng/l ATP for 0.5 and 1 h stagnation, on the 20th of September 2005 in comparison to results from 1st of July 2005. The difference is visible also in the distribution of total and free ATP concentration- in samples from 01/07/05 total and free ATP almost overlap, whereas on 20/09/05 they are separated with 8-20 ng/l ATP difference (see Fig.22 and Fig.23).

On the 01/07/05 a significant decrease of ATP concentration with longer stagnation time have been observed in the galvanized pipe, whereas on 20/09/05 reverse correlation was noticed. Total ATP concentration was gradually increasing with time and after 16 HS reached its maximum, thus 18.67 ng/l ATP. The difference between total and free ATP concentration was also increasing with longer stagnation times in samples from 20/09/05 (see Fig. 24 and Fig.25)

On the 01/07/05 ATP concentrations were decreasing with stagnation time in the polypropylene pipe and total and free ATP concentrations almost overlap. Similarly on the 20/09/05 total and free ATP concentrations were slowly decreasing with time, but unexpectedly total ATP concentration after 8HS slowly started to increase (see Fig.26 and Fig.27).

The overall conclusion for samples from all materials can be that total and free ATP concentrations are very close to each other (sometimes overlap) in the samples of 01/07/05, whereas the difference is larger in the samples of 20/09/05. The explanation can be that on 20/09/05 significant amounts of bacterial ATP has been detected whereas on 01/07/05 there have been mostly free ATP.

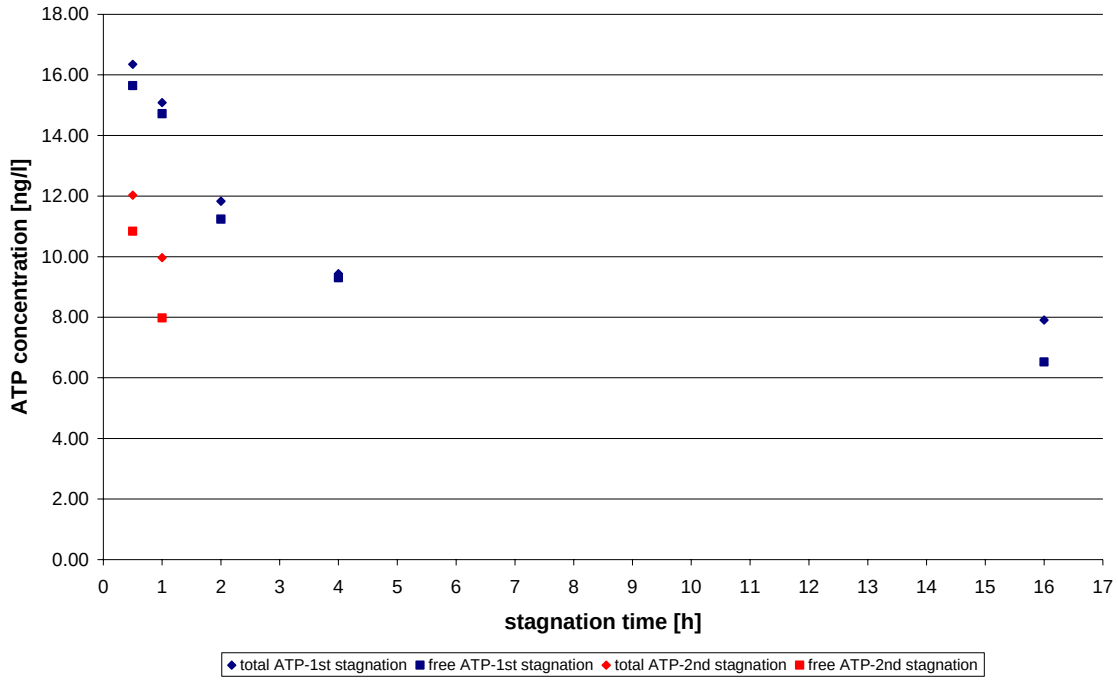

Figure 22 Dependence of ATP concentration on the stagnation time in stainless steel pipe (01/07/05)

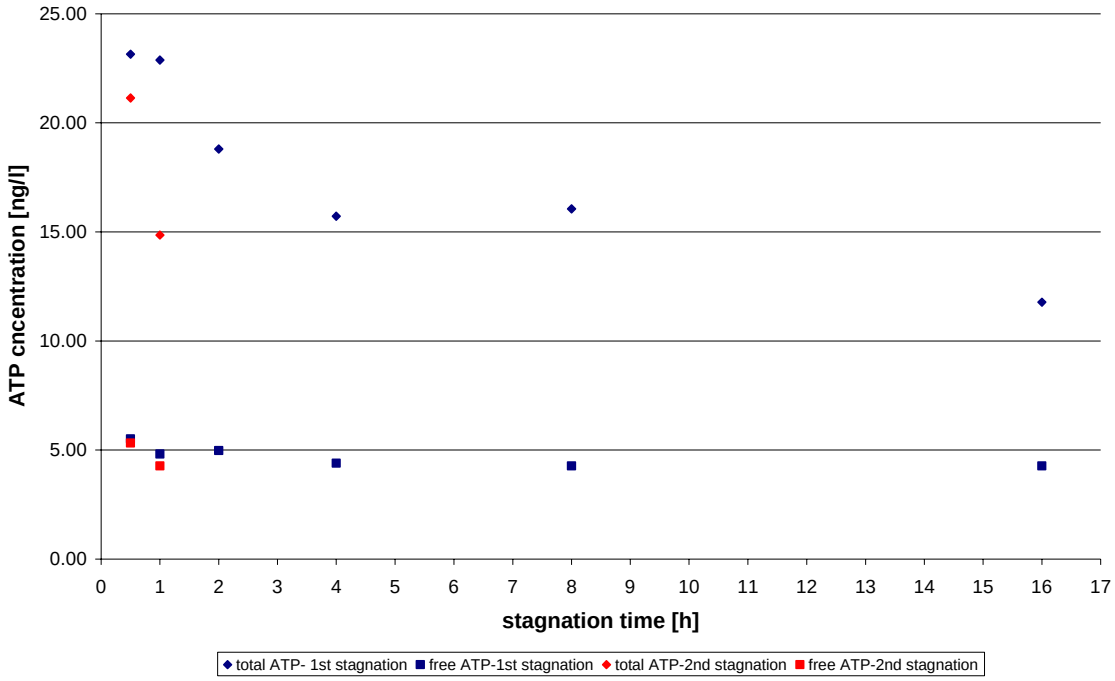

Figure 23 Dependence of ATP concentration on the stagnation time in stainless steel pipe (20/09/05)

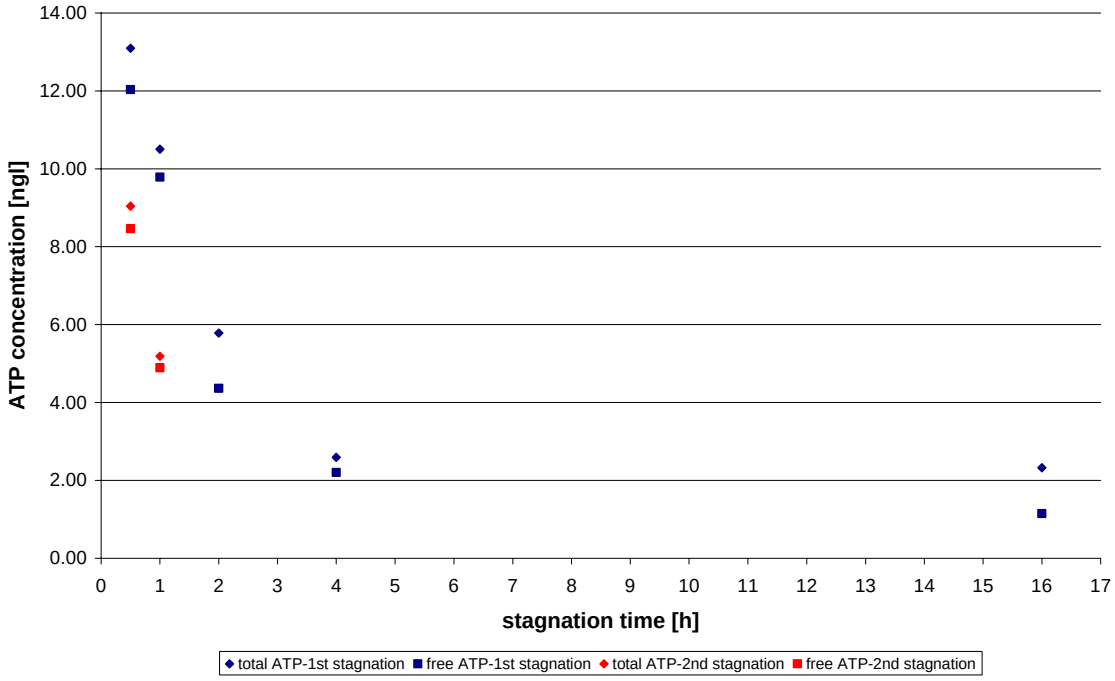

Figure 24 Dependence of ATP concentration on the stagnation time in galvanized pipe (01/07/05)

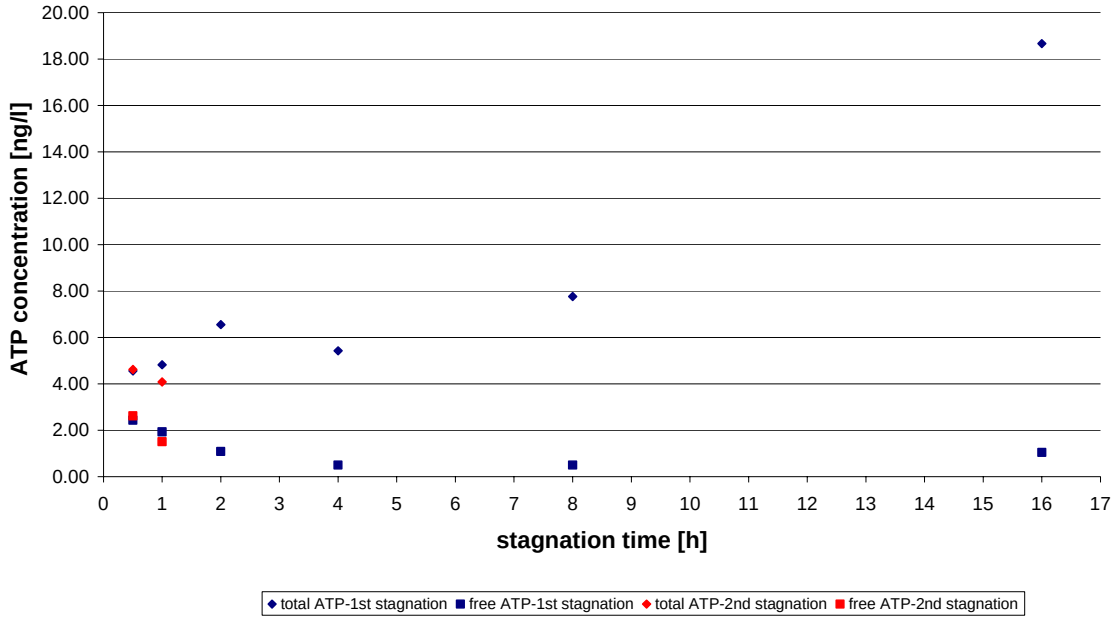

Figure 25 Dependence of ATP concentration on the stagnation time in galvanized pipe (20/09/05)

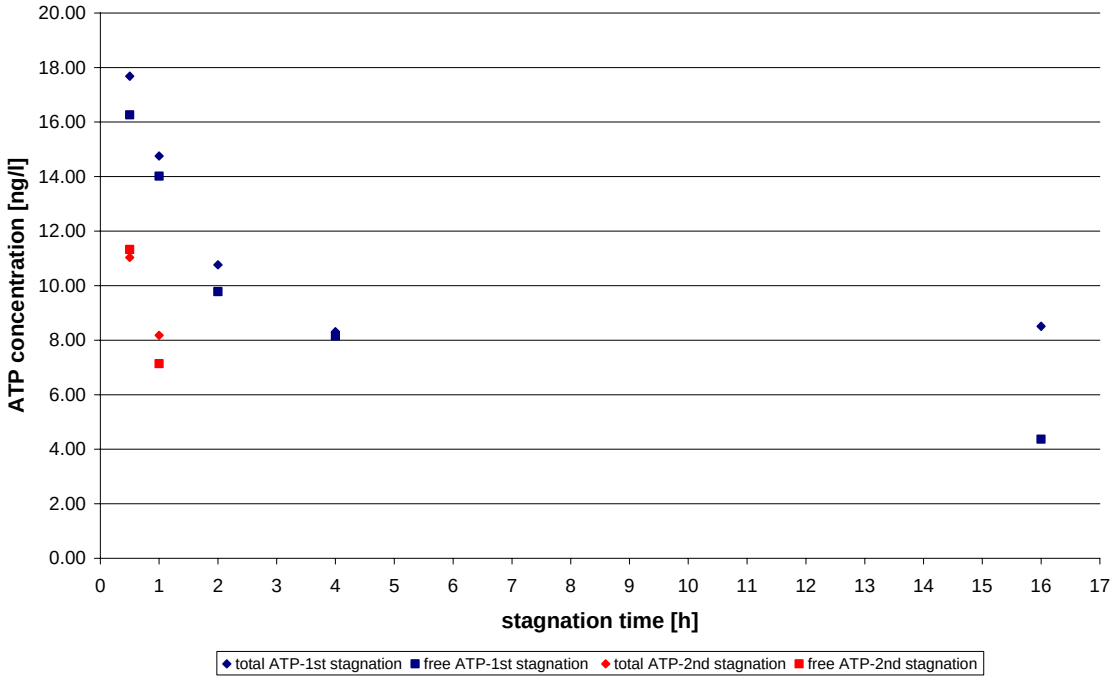

Figure 26 Dependence of ATP concentration on the stagnation time in polypropylene pipe (01/07/05)

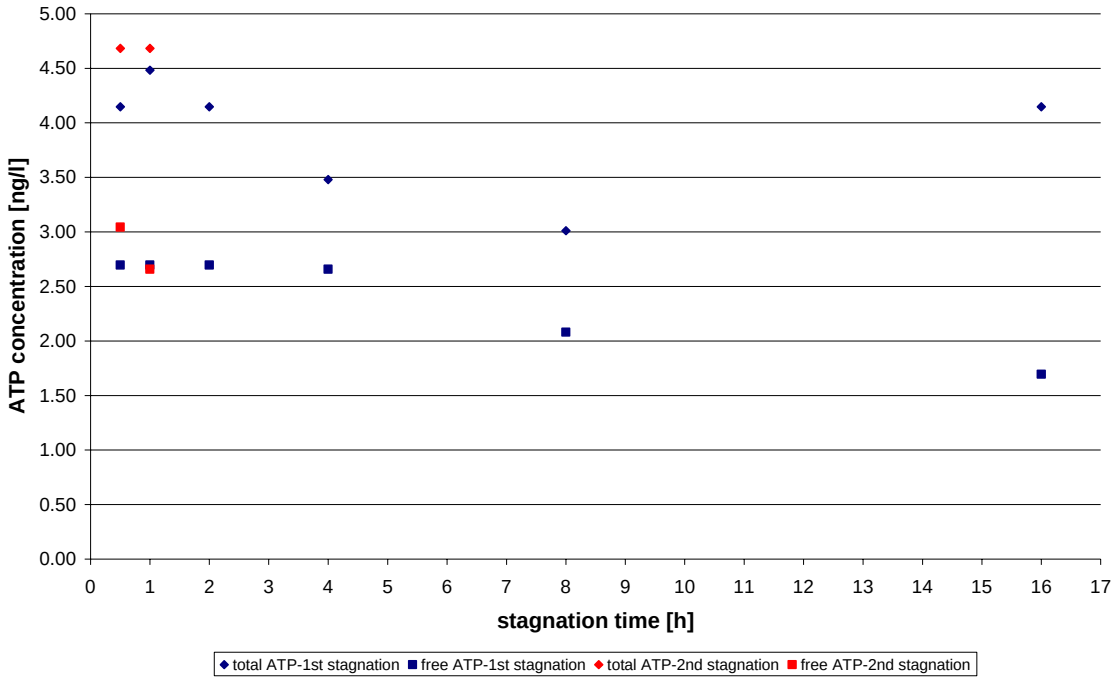

Figure 27 Dependence of ATP concentration on the stagnation time in polypropylene pipe (20/09/05)

Overview of ATP concentrations in tap water samples from different distribution systems

On the 22nd –23rd of August 2005 samples from 6 different places have been collected. The purpose of this survey was to examine microbial activity of tap waters delivered by different distribution systems (see Fig.28). All samples contained 500 ml. FF samples have been taken on 22nd of August after 1 minute of flushing, xHS-1 samples have been taken after 4:40-9:30 hour's stagnation on 23rd of August, xHS-2 samples have been taken directly after xHS-1 samples (see Annex 6.4).

None of the collected samples exceed 10 ng/l ATP, thus the microbial water activity was rather low. In all samples free ATP consisted less than 50% of total ATP. All xHS-1 samples, except the one in Foresteria, contained higher ATP concentrations than FF samples.

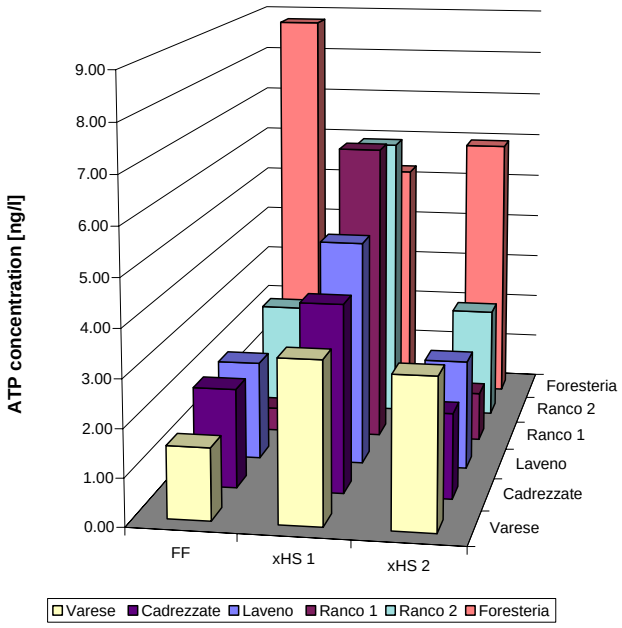

Figure 28 Total ATP concentration in tap water samples

HETEROTROPHIC PLATE COUNTS OF TAP WATER SAMPLES

On the 24th of May 2005 samples have been collected on JRC premises (see list of samples below) and analyzed for total colony count. The sampling procedure started by taking 2 successive 500 ml samples (RDT – random day time), followed by 1 minute of flushing and taking a fully flushed sample (FF). From each numerated sample 1 ml has been put on a petri plate. PCA or R2A medium, kept in a water bath at 45°C, was added and carefully mixed. The dishes were incubated either at 22°C or 37°C. (see Annex 7**).** A control plate has been prepared for each medium and temperature. The samples on PCA and R2A [19] mediums incubated at 37°C were counted after 44 hours. Petri plates with mediums and samples have been put to incubator on 24/05/05 at 19:00 and counted on 26/05/05 at 15:00. The samples on PCA and R2A mediums incubated in 22°C were counted after 68 hours. Mediums with samples have been inserted to incubator on 24/05/05 at 19:00 and counted on 27/05/05 at 15:00.

List of samples collected on the JRC premises:

ED 30, room 004 - Milli-Q water (001)
ED 8, room 006 (old mensa):
a. RDT 1 (002)
b. RDT 2 (003)
c. FF (004)
ED 8A, room 003 (new mensa):
a. RDT 1 (005)
b. RDT 2 (006)
c. FF (007)
ED 15, room 004 (the water production point)- FF (008)
ED 30 A, room E15 (the men's toilet):
a. RDT 1 (009)
b. RDT 2 (010)
c. FF (011)
ED 30, room 012 (the valve used for Dynamic Test Facility):
a. RDT 1 (012)
b. RDT 2 (013)
c. FF (014)

Experiment with total colony counts has been repeated on 31st of May 2005. Newly collected samples (see list of samples below) as previously have been analyzed as above (see Annex 7). The samples on PCA and R2A mediums incubated in 37°C have been inserted to incubator on 01/06/05 at 13:30 and counted on 03/06/05 at 15:00. The samples on PCA and R2A mediums incubated in 22°C have been inserted to incubator on 01/06/05 at 13:30 and counted on 06/06/05 at 10:00.

List of samples collected in Laveno, Leggiunio and on the JRC premises:

private house in Laveno, kitchen:
a. RDT 1 (001)
b. RDT 2 (002)
c. FF (003)
private house in Leggiuno, kitchen:
a. RDT 1 (004)
b. RDT 2 (005)
c. FF (006)

ED 8A, room 003 (new mensa):

a. RDT 1 (007)
b. RDT 2 (008)
c. FF (009)

ED 8, room 006 (old mensa):

a. RDT1 (010)
b. RDT2 (011)
c. FF (012)

ED 15, room 004 (the water production point)- FF (013)

Comparison between HPC and ATP assay

An attempt has been made to compare the numbers of microbial cells counted on the mediums and those calculated from the concentration of ATP. All samples cultivated on mediums have been also analyzed for total and free ATP concentrations. ATP concentrations in the samples have been calculated using method for ATP calculation in the samples. ATP values were calculated in ng/l ATP.

In order to estimate the amount of bacteria from the ATP concentration, the concentration of ATP in the cell was assumed to be 1 fg ATP/cell [9,21] .

Calculated numbers of cells should represent only living bacteria and therefore free ATP concentrations have been subtracted from the total ATP concentrations to receive the microbial ATP concentrations in the samples. Since on the PCA medium microbial growth was rather limited, the comparison has been made for R2A medium at both temperatures.

Results of the comparison between microbial numbers cultivated on R2A mediums and calculated using the ATP assay show that the cell concentration calculated from the ATP concentrations are higher that the real measured colonies (Fig.29, Fig.30, Fig.31, Fig.32). Differences can be due to the presence of non-culturable bacteria. There is also a unknown uncertainty in the assumption of 1 fg ATP per cell.

Detection limit for number of microbial cells counted on mediums has been assessed as 10^3 cells/litre. On R2A medium only one colony have been observed in 1ml in 22^{o}C, but since the number of colonies had to be recalculated and presented in litres the value has been multiplied. Therefore the detection limit went up to 10^3 cells/litre

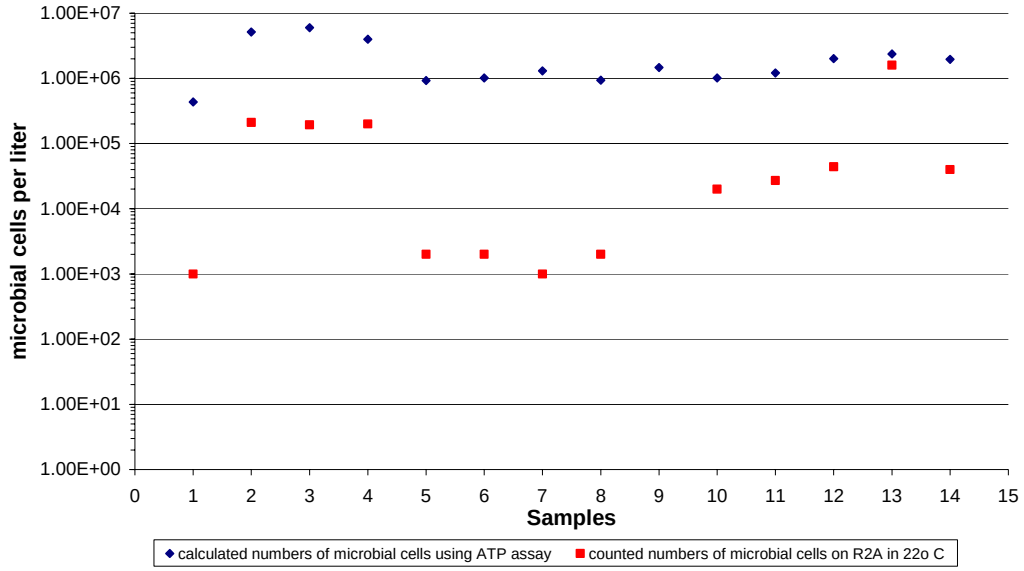

Figure 29 Comparison between number of microbial cells counted on R2A medium in 22^{o} C and calculated using ATP assay (24/05/05)

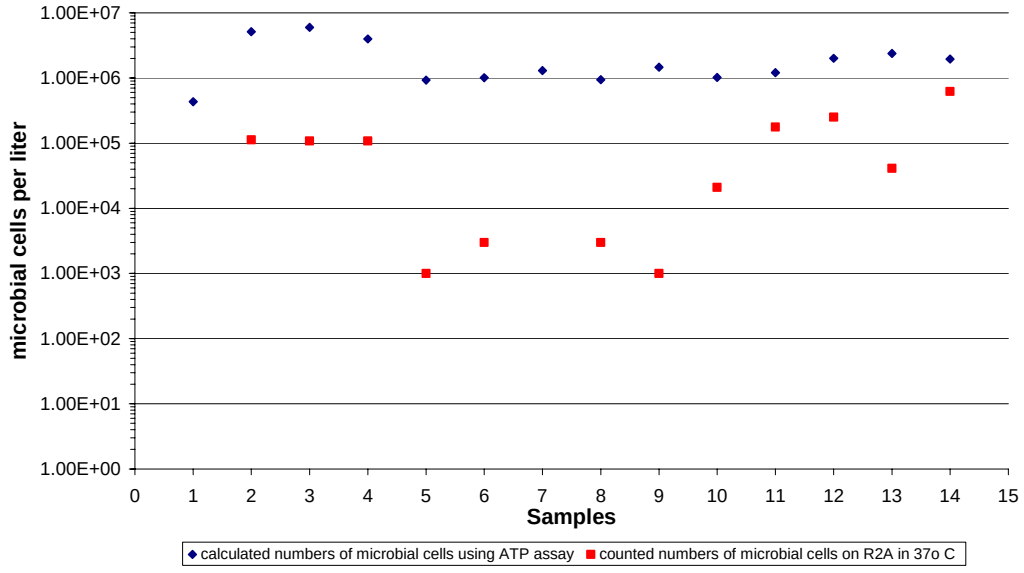

Figure 30 Comparison between number of microbial cells counted on R2A medium in 37^{o} C and calculated using ATP assay (24/05/05)

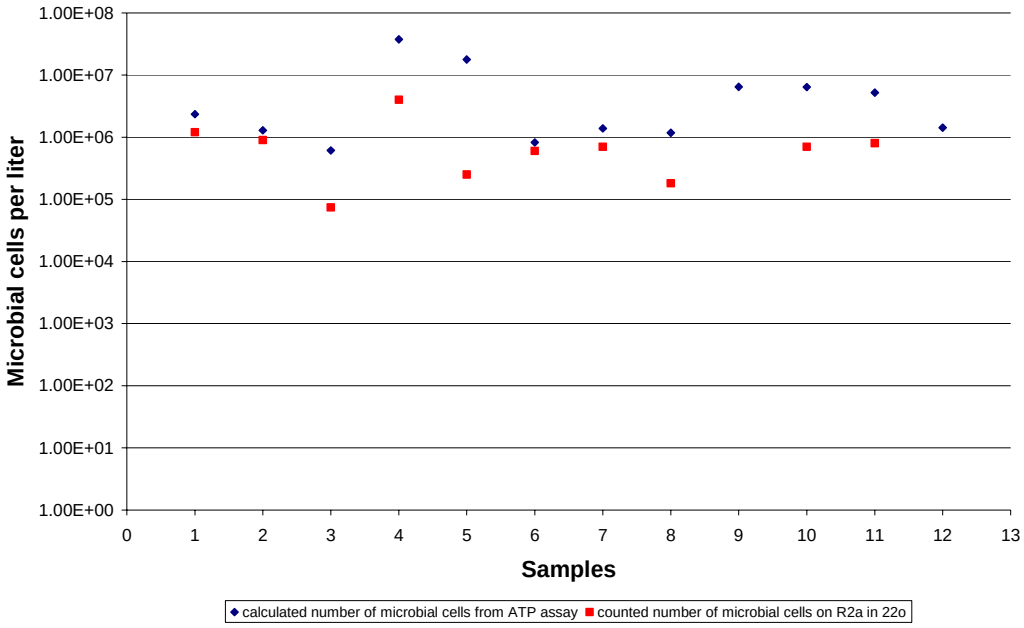

Figure 31 Comparison between number of microbial cells counted on R2A medium in 22° C and calculated using ATP assay (01/06/05)

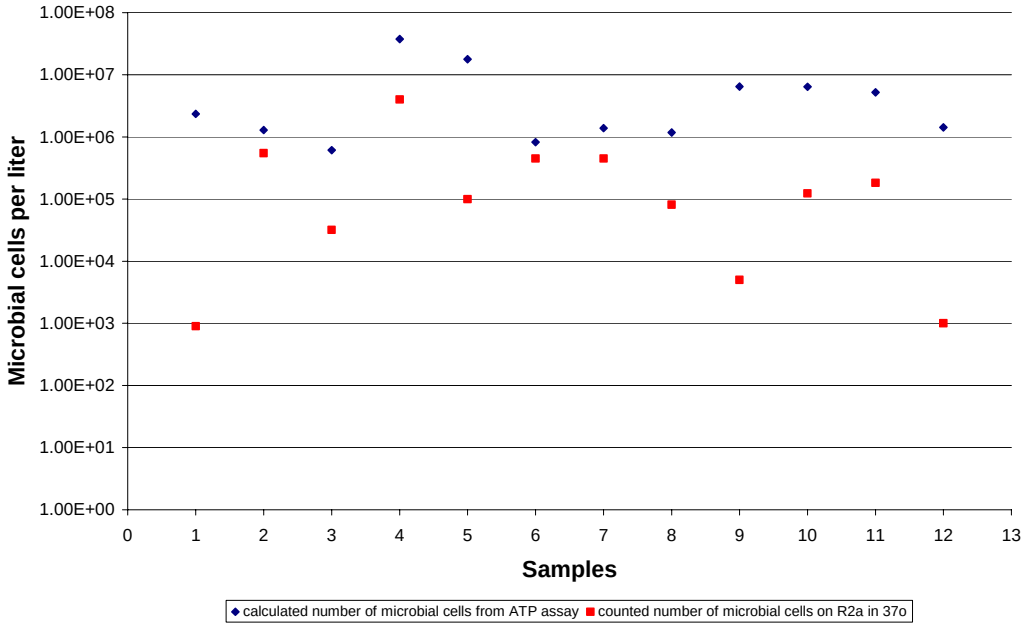

Figure 32 Comparison between number of microbial cells counted on R2A medium in 37° C and calculated using ATP assay (01/06/05)

CONCLUSIONS

1) The detection limit of ATP is determined as 1 ng/l.

2) Polyethylene cuvettes used in all experiments gave a background level of about 40 RLU. The instrument discharged the cuvettes properly even if they were made static. The volume of water sample doesn't have an influence on the background level. Sunlight exposure has an ambiguous influence on RLU values. The background level may vary significantly from batch to batch and it seems better to keep samples in the dark prior to measurement.

3) RLU values of the samples with the same ATP concentrations and the same overall volume are dependent on the concentration of the reagents:
   a) Samples treated with only light generating reagent *LuminATE* give higher response comparing to samples treated additionally with *LuminEX* (quenching effect)
   b) The higher the volume of *LuminATE* used during the measurement the higher will be the RLU value

4) ATP concentrations prepared with tap water show gradual degradation when stored at 24^{o}C. The half-life is 4-5 days and 8-9 days for 20-200 ng/l ATP and 1-20 ng/l ATP concentration, respectively. Half-life for all ATP concentrations prepared with tap water and keep at 4^{o}C is in the range of 8-10 days. ATP concentrations prepared with milli-Q water and kept at 4^{o}C and 24^{o}C are rather stable with time.

5) Tap water samples collected in Leggiuno gradually decrease with longer stagnation times, fully flushed samples and short stagnation time samples contain the highest ATP concentrations. Tap water samples from Laveno show completely reverse distribution, ATP concentrations increase very slowly with stagnation time.

6) Almost all first 500 ml samples (of totally 1 litre of 2 successive RDT samples with defined stagnation time) contain higher ATP concentrations. Leggiuno samples had very similar ATP concentrations.

7) 20 successive 50 ml samples collected in Laveno and Leggiuno confirmed the results of the 500 ml samples. In addition the 50 ml samples show an ATP production in the distribution system near the tap of the house in Laveno. The house in Leggiuno seems to be supplied with drinking water which is rather biologically active.

8) ATP concentrations in all tubes in the Dynamic Test Facility were decreasing with increasing stagnation time. One exception was observed for galvanized steel with a reverse ATP stagnation curve. The first 0.5 and 1 hour stagnation times on a day gave always higher values than the second ones. ATP concentration was also slowly decreasing with time in Leggiuno samples.

9) It is not possible to compare results of tap water samples with results of DTF samples. Samples probably contained different physical and chemical parameters, but real values were not measured. In future experiments they should be taken into account (e.g. it is essential to check chlorine levels in tap water, prior to sampling).

10) Incoming water samples in DTF should be also included in sampling protocol of future experiments (water from the main valve).

11) Control plates (blank) show that mediums with tap water samples have been prepared properly – there weren't any colonies forming.

12) Comparison between numbers of microbial cells counted on R2A mediums and calculated using ATP assay show that results doesn't fit very well. Whereas for some samples the data are comparable, for others the difference in number of microbial cells is within three orders of magnitude higher. The number of microbial cells calculated using ATP concentrations is higher most probably due to the fact that not all bacterial cells were culturable on mediums.

13) On the PCA medium in both temperatures microbiological growth was lower than on the R2A medium, therefore R2A was a better medium for microbiological growth in our samples. The highest bacterial growth was on R2A medium in 22^{o}C, than on R2A medium in 37^{o}C. On PCA medium growth was mostly better in 37^{o} C than in 22^{o}C.

14) ATP can be a good, fast and sensitive indicator of microbiological activity in tap water samples.

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ANNEX 1 DETECTION LIMIT OF ATP

Table 1 detection limit for total ATP

Samples	response RLU	background RLU	average RLU	median RLU	stdev RLU	rstdev %	n
Milli-Q	43	30	45	44	3	6	6
Milli-Q	43	40					
Milli-Q	42	30					
Milli-Q	49	20					
Milli-Q	45	30					
Milli-Q	46	30					
0.2 ng/l	54	40	48	48	6	12	6
0.2 ng/l	48	40					
0.2 ng/l	56	40					
0.2 ng/l	44	60					
0.2 ng/l	41	40					
0.2 ng/l	47	40					
1 ng/l	69	40					
1 ng/l	68	30					
1 ng/l	68	40	65	66	5	8	6
1 ng/l	65	60	65	67	5	8	8
1 ng/l	69	50					
1 ng/l	66	40					
1 ng/l	59	30					
1 ng/l	56	50					
2 ng/l	77	50					
2 ng/l	79	40					
2 ng/l	76	50	79	79	3	3	6
2 ng/l	76	50	79	79	2	3	8
2 ng/l	82	70					
2 ng/l	79	20					
2 ng/l	82	40					
2 ng/l	79	40					
20 ng/l	357	30	360	357	5	1	5
20 ng/l	365	40					
20 ng/l	356	40					
20 ng/l	365	60					
20 ng/l	356	50					
200 ng/l	2118	20	2428	2450	317	13	6
200 ng/l	2081	40					
200 ng/l	2396	40					
200 ng/l	2522	40					
200 ng/l	2504	50					
200 ng/l	2948	40					

ANNEX 2 CALIBRATION DATA

Table 2 Calibration of total ATP on 4 May 2005 in milliQ water

	response RLU	background RLU	linear regression	
Milli-Q	43	30	0-200 ng/l	
Milli-Q	43	40	a=11.864359	b=60.3423
Milli-Q	42	30	0.2685943	21.1596
Milli-Q	49	20	0.9813899	119.218
Milli-Q	45	30	1951.1696	37
Milli-Q	46	30	27731810	525878
0.2 ng/l	54	40		
0.2 ng/l	48	40		
0.2 ng/l	56	40	0.2-200 ng/l	
0.2 ng/l	44	60	a= 11.84	63.996
0.2 ng/l	41	40	0.299	25.624
0.2 ng/l	47	40	0.981	130.02
1 ng/l	69	40	1567	31
1 ng/l	68	30	3E+07	524027
1 ng/l	68	40		
1 ng/l	65	60	1-200 ng/l	
1 ng/l	69	50	a=11.815	b=69.466
1 ng/l	66	40	0.3431	32.487
1 ng/l	59	30	0.9793	144.4
1 ng/l	56	50	1185.6	25
2 ng/l	77	50	2E+07	521319
2 ng/l	79	40		
2 ng/l	76	50		
2 ng/l	76	50		
2 ng/l	82	70		
2 ng/l	79	20		
2 ng/l	82	40		
2 ng/l	79	40		
20 ng/l	357	30		
20 ng/l	365	40		
20 ng/l	356	40		
20 ng/l	365	60		
20 ng/l	356	50		
200 ng/l	2118	20		
200 ng/l	2081	40		
200 ng/l	2396	40		
200 ng/l	2522	40		
200 ng/l	2504	50		
200 ng/l	2948	40		

Table 3 Calibration of free and total ATP on 24 May 2005 in milliQ water

	Response RLU	Background RLU	linear regression 0-200 ng/l		linear regression 1-200 ng/l	
Total ATP						
Milli-Q	60	50	a=15.45174857	b=69.5520138	a=15.435701	b=72.45968
Milli-Q	63	40	0.112518983	10.1148245	0.1335739	13.424843
1 ng/l	84	70	0.999575965	27.7715451	0.9995509	31.593973
1 ng/l	82	60	18858.34895	8	13353.939	6
2 ng/l	105	40	14544666.03	6170.06974	13329623	5989.0748
2 ng/l	100	50				
20 ng/l	390	40				
20 ng/l	385	60				
200 ng/l	3105	30				
200 ng/l	3213	40				
Free ATP						
Milli-Q	62	40	a=23.36544295	b=62.0012443	a=23.36843	b=61.460028
Milli-Q	65	40	0.192624015	17.3158169	0.2320299	23.320159
1 ng/l	85	30	0.999456591	47.5427911	0.9994088	54.881571
1 ng/l	87	70	14713.87888	8	10143.111	6
2 ng/l	112	40	33258030.36	18082.5359	30550916	18071.921
2 ng/l	109	50				
20 ng/l	516	40				
20 ng/l	534	30				
200 ng/l	4641	40				
200 ng/l	4830	40				

Table 4 Calibration of free and total ATP on 31 May 2005 in milliQ water

	Response RLU	Background RLU	linear regression 0-200 ng/l	
Total ATP				
Milli-Q	48	40	a=15.66630115	b=45.28296869
1 ng/l	63	30	0.019123675	1.719110924
2 ng/l	76	30	0.99999553	3.337571613
20 ng/l	354	40	671104.6501	3
200 ng/l	3179	40	7475692.582	33.41815281
Free ATP				
Milli-Q	44	40	a=24.88962941	b=32.5225285
1 ng/l	66	40	0.100239386	9.010957687
2 ng/l	86	30	0.999951344	17.49434325
20 ng/l	504	50	61653.83083	3
200 ng/l	5013	30	18869281.04	918.1561367

Table 5 Calibration of free and total ATP on 9 June 2005 in milliQ water, sterile water and water of water supply

	Response RLU	Background RLU	linear regression	1-2000 ng/l	linear regression	1-200 ng/l
Total ATP						
water supply	140	40				
1 ng/l	180	40	a=18.6765759	b=196.5943	a=19.63201	b=146.7656
2 ng/l	190	50	0.05364739	48.22542	0.101884	10.23983
20 ng/l	520	50	0.99997525	93.72275	0.999946	17.04012
200 ng/l	4075	30	121198.543	3	37129.5	2
2000 ng/l	37536	40	1064602469	26351.86	10781138	580.7317
Free ATP						
water supply	181	40				
1 ng/l	224	40	a=26.6080525	b=278.2599	a=28.16252	b=197.1893
2 ng/l	248	40	0.08637311	77.64367	0.040081	4.028306
20 ng/l	768	40	0.99996839	150.8951	0.999996	6.703513
200 ng/l	5829	50	94900.6126	3	493711.7	2
2000 ng/l	53472	40	2160822685	68307.97	22185965	89.87416
Total ATP						
sterile water	40	40				
1 ng/l	65	50	a=19.3941273	b=-7.22902	a=18.07531	b=61.55122
2 ng/l	87	40	0.07405826	66.57344	0.141242	14.19557
20 ng/l	451	40	0.99995626	129.3808	0.999878	23.62289
200 ng/l	3674	40	68579.3626	3	16377.25	2
2000 ng/l	38800	40	1147977667	50218.21	9139173	1116.081
Free ATP						
sterile water	43	40				
1 ng/l	71	40	a=29.7092617	b=-129.538	a=26.58053	b=33.63549
2 ng/l	98	40	0.17399358	156.4086	0.122153	12.277
20 ng/l	541	50	0.99989711	303.9693	0.999958	20.43019
200 ng/l	5352	30	29155.2846	3	47349.74	2
2000 ng/l	59334	40	2693870231	277192	19763434	834.7853
Total ATP						
Milli-Q	63	50				
1 ng/l	82	50	a=19.2604219	b=88.21643	a=19.61213	b=69.87367
2 ng/l	94	40	0.02192319	19.70748	0.127431	12.8074
20 ng/l	487	30	0.99999611	38.30012	0.999916	21.31283
200 ng/l	3990	70	771834.459	3	23686.59	2
2000 ng/l	38604	40	1132203635	4400.699	10759318	908.4732
Free ATP						
Milli-Q	54	40				
1 ng/l	97	40	a=28.4461729	b=186.6315	a=30.87727	b=59.84201
2 ng/l	112	40	0.13505199	121.4027	0.050854	5.111033
20 ng/l	681	40	0.99993238	235.9378	0.999995	8.505282
200 ng/l	6235	40	44365.5354	3	368668.1	2
2000 ng/l	57044	40	2469679763	166999.9	26669388	144.6796

Table 6 Calibration of free and total ATP on 1 July 2005 in sterile water and water of water supply

	response RLU	Background RLU	linear regression	1-200 ng/l
Total ATP				
water supply	384	30		
1 ng/l	426	30	a=15.0451	b=390.738308
2 ng/l	432	40	0.17656	17.7449493
20 ng/l	657	30	0.99972	29.5294111
200 ng/l	3403	60	7261.28	2
2000 ng/l	32368	50	6331733	1743.97224
Free ATP				
water supply	589	50		
1 ng/l	614	40	a=22.6876	b=582.664432
2 ng/l	627	40	0.05132	5.15832807
20 ng/l	1028	60	0.99999	8.58398563
200 ng/l	5121	40	195405	2
2000 ng/l	50775	40	1.4E+07	147.369619
Total ATP				
sterile water	40	40		
1 ng/l	72	60	a=16.1234	b=61.8726171
2 ng/l	85	50	0.08446	8.48838252
20 ng/l	401	40	0.99995	14.1255369
200 ng/l	3285	60	36444.9	2
2000 ng/l	31389	40	7271874	399.061587
Free ATP				
sterile water	41	30		
1 ng/l	83	60	a=23.1696	b=77.7966503
2 ng/l	96	40	0.25781	25.9111139
20 ng/l	592	40	0.99975	43.1187443
200 ng/l	4707	40	8076.79	2
2000 ng/l	55448	40	1.5E+07	3718.45223

Table 7 Calibration of free and total ATP on 16 August 2005 in water of water supply

	Response RLU	Background RLU	linear regression 1-200 ng/l	
Total ATP				
200 ng/l	3158	40	a=15.0945295	b=140.9799805
20 ng/l	463	40	0.10206424	10.25796389
2 ng/l	164	50	0.999908568	17.07030135
1 ng /l	145	50	21872.17383	2
water prod.				
point	55	50	6373446.21	582.7903763
sterile water	68	30		
Free ATP				
200 ng/l	5243	40	a=25.44261826	b=155.5740319
20 ng/l	675	40	0.143408974	14.41331534
2 ng/l	224	40	0.999936462	23.98523127
1 ng/l	154	40	31475.37745	2
water prod.				
point	164	30	18107511.42	1150.582638
sterile water	62	40		

Table 8 Calibration of free and total ATP on 23 August 2005 in water of water supply

	Response RLU	Background RLU	linear regression 1-200 ng/l	
total ATP				
sterile water	58	70		
water supply	148	70	a=13.32697893	b=143.2709244
1 ng/l	154	40	0.036439662	3.662367268
2 ng/l	166	40	0.999985048	6.094553812
20 ng/l	417	40	133756.4564	2
200 ng/l	2808	50	4968194.463	74.28717234
free ATP				
sterile water	58	30		
water supply	162	40	a=23.19182955	b=159.3055027
1 ng/l	173	50	0.051545307	5.180559785
2 ng/l	209	30	0.999990121	8.620981478
20 ng/l	630	40	202437.8708	2
200 ng/l	4797	40	15045450.11	148.6426433

Table 9 Calibration of free and total ATP on 20 September 2005 in water of water supply

	Response RLU	Background RLU	linear regression 1-200 ng/l	
Total ATP				
sterile water	40	20		
wat.prod.point	126	20	a=14.94665344	b=139.7240707
1 ng/l	168	40	0.083828756	8.425207049
2 ng/l	155	40	0.999937093	14.02040647
20 ng/l	440	30	31790.83131	2
200 ng/l	3129	40	6249180.856	393.1435951
2 mg/l	99999999	30		
			Concentration ng/l	
2 ng/l ▲	308	40	12.18	
2 ng/l ▲	114	40	-0.80	
Free ATP				
sterile water	46	40	a=25.95866513	b=177.054419
wat.prod.point	154	50	0.150724126	15.14852447
1 ng/l	187	20	0.999932578	25.20869449
2 ng/l	218	40	29661.91363	2
20 ng/l	726	40	18849501.79	1270.956556
200 ng/l	5366	30		
2 mg/l	99999999	40		
			Concentration ng/l	
2 ng/l ▲	209	70	2.12	
2 ng/l ▲	120	40	-1.31	

▲ these ATP concentrations have been inserted and measured between samples for control.

Table 10 Calibration of total ATP on 14 October 2005 in milliQ water and water of water supply

	Response RLU	Background RLU	linear regression 1-200 ng/l	
200 ng/l water prod. point	2891	20	a=13.883029	b=115.52113
20 ng/l water prod. point	405	40	0.06675031	6.7087378
2 ng/l water prod. point	143	20	0.99995377	11.164026
1 ng/l water prod. point	119	30	43257.5533	2
water prod. point	116	40	5391425.73	249.27095
			Calculated concentration ng/l	
2 ng/l water prod. point ▲	159	40		8.57
20 ng/l water prod. point ▲	397	40		25.71
200 ng/l milli-Q water	2980	40	a=14.6885898	b=42.361119
20 ng/l milli-Q water	337	40	0.00483092	0.4855318
2 ng/l milli-Q water	71	40	0.99999978	0.8079745
1 ng/l milli-Q water	57	40	9244853.44	2
milli-Q water	47	20	6035251.44	1.3056457
			Calculated concentration ng/l	
2 ng/l milli-Q water ▲	69	40		1.97
2 ng/l milli-Q water ▲	68	40		1.91
2 ng/l milli-Q water ▲	73	20		2.25
20 ng/l milli-Q water ▲	350	70		21.10

▲ these ATP concentrations have been inserted and measured between samples for control.

ANNEX 3 RLU RESPONSE

Table 11 Effect of the volume of water in the cuvette on the background response

	Response RLU	Background RLU	average RLU	stdev RLU	rstdev %	n
First batch						
100 µl milli-Q water	37	30	37.67	2.503	6.646	6
100 µl milli-Q water	38	20				
100 µl milli-Q water	39	40				
100 µl milli-Q water	33	40				
100 µl milli-Q water	40	70				
100 µl milli-Q water	39	30				
200 µl milli-Q water	40	50	38.67	1.506	3.894	6
200 µl milli-Q water	37	50				
200 µl milli-Q water	40	40				
200 µl milli-Q water	38	40				
200 µl milli-Q water	40	50				
200 µl milli-Q water	37	30				
300 µl milli-Q water	41	40	36.83	3.488	9.47	6
300 µl milli-Q water	34	40				
300 µl milli-Q water	33	30				
300 µl milli-Q water	35	40				
300 µl milli-Q water	37	60				
300 µl milli-Q water	41	40				
Second batch						
100 µl milli-Q water	44	60	44.33	2.34	5.27	6
100 µl milli-Q water	46	40				
100 µl milli-Q water	44	40				
100 µl milli-Q water	40	40				
100 µl milli-Q water	46	60				
100 µl milli-Q water	46	40				

Table 12 effect of sunlight on the background response of cuvettes filled with 100 µl milliQ water

Exposure time	Response RLU	Background RLU	average RLU	stdev RLU	rstdev %	n
0 min	44	60	44.33	2.34	5.27	6
0 min	46	40				
0 min	44	40				
0 min	40	40				
0 min	46	60				
0 min	46	40				
2 min	51	40	59.00	5.62	9.53	6
2 min	67	60				
2 min	56	60				
2 min	57	50				
2 min	60	60				
2 min	63	50				
2 min light-2 min dark	42	40	40.60	1.95	4.80	5
2 min light-2 min dark	40	30				
2 min light-2 min dark	38	20				
2 min light-2 min dark	40	40				
2 min light-2 min dark	43	40				
5 min	73	70	59.50	7.26	12.20	6
5 min	62	70				
5 min	55	60				
5 min	53	60				
5 min	57	50				
5 min	57	40				
10 min	61	80	60.67	10.50	17.31	6
10 min	68	70				
10 min	59	70				
10 min	54	30				
10 min	76	60				
10 min	46	70				
20 min	52	60	50.33	6.22	12.35	6
20 min	54	60				
20 min	47	30				
20 min	46	50				
20 min	43	40				
20 min	60	40				
40 min	65	80	66.17	6.82	10.31	6
40 min	67	70				
40 min	79	70				
40 min	61	50				
40 min	60	60				
40 min	65	70				
80 min	70	50	64.67	6.19	9.57	6
80 min	59	70				
80 min	65	70				
80 min	72	80				
80 min	56	70				
80 min	66	70				
160 min	66	70	62.33	3.14	5.04	6
160 min	66	80				
160 min	61	70				
160 min	62	90				
160 min	61	60				
160 min	58	70				

Exposure time	Response RLU	Background RLU	average RLU	stdev RLU	rstdev %	n
320 min	47	40	48.33	3.78	7.81	6
320 min	54	50				
320 min	51	40				
320 min	45	50				
320 min	44	40				
320 min	49	60				

Table 13 Effect of sunlight on the background response of cuvettes filled with 100 µl milliQ water made anti-static by ethanol

Exposure time	Response RLU	Background RLU	average RLU	stdev RLU	rstdev %	n
0 min	52	70	50.33	4.27	8.49	6
0 min	49	50				
0 min	51	40				
0 min	49	60				
0 min	57	40				
0 min	44	40				
2 min	52	50	49.67	4.55	9.15	6
2 min	53	50				
2 min	55	60				
2 min	43	50				
2 min	46	50				
2 min	49	30				
2 min light-2 min dark	40	20	42.17	2.23	5.29	6
2 min light-2 min dark	43	50				
2 min light-2 min dark	42	40				
2 min light-2 min dark	46	50				
2 min light-2 min dark	40	60				
2 min light-5 min dark	45	60	45.33	2.58	5.70	6
2 min light-5 min dark	46	30				
2 min light-5 min dark	43	40				
2 min light-5 min dark	49	40				
2 min light-5 min dark	47	40				
2 min light-5 min dark	42	40				
0 min made static	41	30	46.00	3.22	7.01	6
0 min made static	49	40				
0 min made static	48	60				
0 min made static	47	60				
0 min made static	43	40				
0 min made static	48	40				

Table 14 Effect of LuminEx on response of a 200 ng/l solution of ATP

Sample	Response RLU	Background RLU	average RLU	stdev RLU	rstdev %	n
100µl LuminEX	2833	50	2774.33	155.5	5.61	3
100µl LuminEX	2892	50				
100µl LuminEX	2598	50				
100µl H2O	3010	70	3125.67	105.3	3.37	3
100µl H2O	3151	50				
100µl H2O	3216	40				

Table 15 Effect of concentration of LuminATE on the response of mixture of 100 µl of 200 ng/l ATP, while the total volume remains constant

	Response RLU	Background RLU
150 µl H2O+50 µl LuminATE	1150	60
100 µl H2O+100 µl LuminATE	3189	70
50 µl H2O+150 µl LuminATE	2004*	40
150µl H2O +50 µl LuminATE	1135	40
100µl H2O+100 µl LuminATE	3685	40
50µl H2O+150 µl LuminATE	4708	40

* the response in this sample is not correct, since a wrong method had been used

ANNEX 4 METHODS FOR ATP CALCULATION IN THE SAMPLES

Table 16 Samples of 09/06/05 calculated from calibration curve X = (y-b)/a

	Response	Background	0-2000 ng/l			0-200 ng/l		
	RLU	RLU	supply ng/l	sterile ng/l	Milli-Q ng/l	supply ng/l	sterile ng/l	Milli-Q ng/l
Total ATP								
001	98	40	-4.64	4.92	0.78	-2.39	2.33	1.53
002	87	50	-5.23	4.35	0.21	-2.95	1.72	0.97
003	78	40	-5.71	3.89	-0.26	-3.41	1.22	0.51
004	86	40	-5.29	4.30	0.16	-3.00	1.67	0.91
005	148	50	-1.97	7.50	3.38	0.15	5.09	4.07
MilliQ	49	30	-7.27	2.39	-1.76	-4.89	-0.38	-0.97
Free ATP								
001	101	50	-5.89	6.55	-2.03	-3.26	2.44	1.38
002	94	30	-6.16	6.31	-2.28	-3.51	2.18	1.16
003	88	30	-6.38	6.11	-2.49	-3.72	1.95	0.96
004	82	40	-6.61	5.91	-2.70	-3.93	1.73	0.77
005	57	60	-7.55	5.07	-3.58	-4.82	0.79	-0.04
MilliQ	51	70	-7.77	4.86	-3.79	-5.03	0.56	-0.24
				1-2000 ng/l			1-200 ng/l	
Total ATP								
001	98	40	-5.28	5.43	0.51	-2.48	2.02	1.43
002	87	50	-5.87	4.86	-0.06	-3.04	1.41	0.87
003	78	40	-6.35	4.39	-0.53	-3.50	0.91	0.41
004	86	40	-5.92	4.81	-0.12	-3.10	1.35	0.82
005	148	50	-2.60	8.00	3.10	0.06	4.78	3.98
MilliQ	49	30	-7.90	2.90	-2.04	-4.98	-0.69	-1.06
Free ATP								
001	101	50	-6.66	7.76	-3.01	0.37	2.53	1.33
002	94	30	-6.92	7.52	-3.26	0.33	2.27	1.11
003	88	30	-7.15	7.32	-3.47	0.30	2.05	0.91
004	82	40	-7.38	7.12	-3.68	0.27	1.82	0.72
005	57	60	-8.32	6.28	-4.56	0.15	0.88	-0.09
MilliQ	51	70	-8.54	6.08	-4.77	0.12	0.65	-0.29

Table 17 Samples of 09/06/05 calculated from slope calibration curve "a" and the background measured: X = (y-background)/a

	Response	Background	1-200 ng/l		
	RLU	RLU	supply ng/l	sterile ng/l	Milli-Q ng/l
Total ATP					
001	98	40	4.99	5.42	5.00
002	87	50	4.43	4.81	4.44
003	78	40	3.97	4.32	3.98
004	86	40	4.38	4.76	4.39
005	148	50	7.54	8.19	7.55
MilliQ	49	30	2.50	2.71	2.50
Free ATP					
001	101	50	3.59	3.80	3.27
002	94	30	3.34	3.54	3.04
003	88	30	3.12	3.31	2.85
004	82	40	2.91	3.08	2.66
005	57	60	2.02	2.14	1.85
MilliQ	51	70	1.81	1.92	1.65

Table 18 Samples of 09/06/05 calculated from slope calibration curve "a" and the average value of the empty cuvettes: X = (y-factor)/a

	Response	Background	1-200 ng/l		
	RLU	RLU	supply ng/l	sterile ng/l	Milli-Q ng/l
Total ATP					
001	98	40	2.95	3.21	2.96
002	87	50	2.39	2.60	2.40
003	78	40	1.94	2.10	1.94
004	86	40	2.34	2.54	2.35
005	148	50	5.50	5.97	5.51
MilliQ	49	30	0.46	0.50	0.46
Free ATP					
001	101	50	2.17	2.29	1.98
002	94	30	1.92	2.03	1.75
003	88	30	1.70	1.81	1.55
004	82	40	1.49	1.58	1.36
005	57	60	0.60	0.64	0.55
MilliQ	51	70	0.39	0.41	0.36

ANNEX 5 ATP STABILITY AND DEGRADATION

Table 19 ATP degradation for four days (16-20/09/05)

	Response RLU	Bkgnd RLU	concentration ng/l	Corrected response* RLU	Corrected concentration* ng/l
total ATP, 24°					
wat.prod.point	110	40	4.68		
1ng/l water.prod.point	109	40	4.62	-1	-0.07
2 ng/l water.prod.point	117	40	5.15	7	0.47
20 ng/l water.prod.point	234	40	12.98	124	8.30
200 ng/l water.prod.point	1727	40	112.87	1617	108.18
milli-Q water	46	40	0.40		
1 ng/l milli-Q water	61	20	1.40	15	1.00
2 ng/l milli-Q water	79	50	2.61	33	2.21
20 ng/l milli-Q water	350	40	20.74	304	20.34
200 ng/l milli-Q water	3568	40	236.04	3522	235.64
2 mg/l milli-Q water	1E+08	40	6690458.13		
free ATP, 24°					
wat.prod.point	142	40	3.93		
1 ng/l water.prod.point	156	40	4.47	14	0.54
2 ng/l water.prod.point	159	30	4.58	17	0.65
20 ng/l water.prod.point	331	40	11.21	189	7.28
200 ng/l water.prod.point	2988	30	113.57	2846	109.64
milli-Q water	50	30	0.39		
1 ng/l milli-Q water	71	40	1.19	21	0.81
2 ng/l milli-Q water	98	40	2.23	48	1.85
20 ng/l milli-Q water	523	40	18.61	473	18.22
200 ng/l milli-Q water	5740	30	219.58	5690	219.19
Total ATP, 4°					
water prod.point	112	30	4.82		
1ng/l water.prod.point	129	30	5.95	17	1.14
2 ng/l water.prod.point	114	50	4.95	2	0.13
20 ng/l water.prod.point	320	40	18.73	208	13.92
200 ng water.prod.point	2517	40	165.72	2405	160.91
milli-Q water	42	40	0.13		
1 ng/l milli-Q water	135	40	6.36	93	6.22
2 ng/l milli-Q water	82	40	2.81	40	2.68
20 ng/l milli-Q water	384	20	23.02	342	22.88
200 ng/l milli-Q water	3303	40	218.31	3261	218.18
2 mg/l milli-Q water	1E+08	40	6690458.13		
Free ATP, 4°					
water prod. point	128	70	3.39		
1 ng/l water.prod.point	132	40	3.54	4	0.15
2 ng/l water.prod.point	154	40	4.39	26	1.00
20 ng/l water.prod.point	492	40	17.41	364	14.02
200 ng/l water.prod.point	4107	30	156.67	3979	153.28
milli-Q water	46	40	0.23		
1 ng/l milli-Q water	68	40	1.08	22	0.85
2 ng/l milli-Q water	104	40	2.47	58	2.23
20 ng/l milli-Q water	531	20	18.91	485	18.68
200 ng/l milli-Q water	5291	30	202.28	5245	202.05

* correction for the ATP content of the water of the water supply or milliQ water

Table 20 ATP degradation experiments in the period of 4-14/10/05

	Response RLU	Bkgnd RLU	Concentration ng/l	Degradation time days	Corrected response* RLU	corrected concentration* ng/l
Total ATP, 24°, 13/10/05						
200 ng/l water prod. point	2420	40	171.43	1	2294	165.24
20 ng/l water prod. point	381	30	24.56	1	255	18.37
2 ng/l water prod. point	154	40	8.21	1	28	2.02
1 ng/l water prod. point	146	30	7.64	1	20	1.44
water prod. point	126	50	6.19	1		
200 ng/l milli-Q water	3301	20	222.01	1	3254	221.53
20 ng/l milli-Q water	381	60	23.22	1	334	22.74
2 ng/l milli-Q water	77	20	2.52	1	30	2.04
1 ng/l milli-Q water	64	40	1.63	1	17	1.16
milli-Q water	47	40	0.48	1		
Total ATP, 4°, 13/10/05						
200 ng/l water prod. point	2865	50	203.49	1	2736	197.08
20 ng/l water prod. point	372	40	23.91	1	243	17.50
2 ng/l water prod. point	162	30	8.79	1	33	2.38
1 ng/l water prod. point	136	50	6.91	1	7	0.50
water prod. point	129	20		1		
200 ng/l milli-Q water	3158	30	212.27	1	3121	212.48
20 ng/l milli-Q water	359	30	21.72	1	322	21.92
2 ng/l milli-Q water	74	40	2.31	1	37	2.52
1 ng/l milli-Q water	52	30	0.82	1	15	1.02
milli-Q water	37	40	-0.20	1		
Total ATP, 24°, 10/10/05						
200 ng/l water prod. point	1715	30	120.65	4	1619	116.62
20 ng/l water prod. point	250	40	15.13	4	154	11.09
2 ng/l water prod. point	112	40	5.19	4	16	1.15
1 ng/l water prod. point	108	40	4.90	4	12	0.86
water prod. point	96	40	4.03	4		
200 ng/l milli-Q water	3195	30	214.79	4	3152	214.59
20 ng/l milli-Q water	392	40	23.96	4	349	23.76
2 ng/l milli-Q water	76	30	2.45	4	33	2.25
1 ng/l milli-Q water	55	40	1.02	4	12	0.82
milli-Q water	43	60	0.20	4		
Total ATP, 4°, 10/10/05						
200 ng/l water prod. point	2456	10	174.03	4	2346	168.98
20 ng/l water prod. point	315	40	19.81	4	205	14.77
2 ng/l water prod. point	116	40	5.47	4	6	0.43
1 ng/l water prod. point	118	40	5.62	4	8	0.58
water prod. point	110	40	5.04	4		
200 ng/l milli-Q water	3410	40	229.43	4	3370	229.43
20 ng/l milli-Q water	413	30	25.39	4	373	25.39
2 ng/l milli-Q water	83	30	2.93	4	43	2.93
1 ng/l milli-Q water	54	20	0.95	4	14	0.95
milli-Q water	40	30	0.00	4		

Table 20 continued

	Response RLU	Bkgnd RLU	Concentration ng/l	Degradation time days	Corrected response* RLU	corrected concentration* ng/l
Total ATP, 24°, 6/10/05						
200 ng/l water prod. point	949	70	65.48	8	884	63.67
20 ng/l water prod. point	142	40	7.35	8	77	5.55
2 ng/l water prod. point	86	40	3.31	8	21	1.51
1 ng/l water prod. point	82	30	3.03	8	17	1.22
water prod. point	65	50	1.80	8		
200 ng/l milli-Q water	3313	40	222.83	8	3276	223.03
20 ng/l milli-Q water	321	40	19.13	8	284	19.33
2 ng/l milli-Q water	68	20	1.91	8	31	2.11
1 ng/l milli-Q water	55	40	1.02	8	18	1.23
milli-Q water	37	40	-0.20	8		
Total ATP, 4°, 6/10/05						
200 ng/l water prod. point	1441	20	100.91	8	1367	98.47
20 ng/l water prod. point	193884	30	13962.66	8	2E+05	13960.21
2 ng/l water prod. point	123	40	5.98	8	49	3.53
1 ng/l water prod. point	52	50	0.86	8	-22	-1.58
water prod. point	74	20	2.45	8		
200 ng/l milli-Q water	3232	50	217.31	8	3196	217.58
20 ng/l milli-Q water	349	40	21.04	8	313	21.31
2 ng/l milli-Q water	70	20	2.04	8	34	2.31
1 ng/l milli-Q water	57	40	1.16	8	21	1.43
milli-Q water	36	30	-0.27	8		
Total ATP, 24°, 5/10/05						
200 ng/l water prod. point	1062	40	73.62	9	983	70.81
20 ng/l water prod. point	173	50	9.58	9	94	6.77
2 ng/l water prod. point	96	40	4.03	9	17	1.22
1 ng/l water prod. point	56	30	1.15	9	-23	-1.66
water prod. point	79	40	2.81	9		
200 ng/l milli-Q water	3448	30	232.02	9	3401	231.54
20 ng/l milli-Q water	79	40	2.66	9	32	2.18
2 ng/l milli-Q water	70	40	2.04	9	23	1.57
1 ng/l milli-Q water	80	40	2.72	9	33	2.25
milli-Q water	47	40	0.48	9		
Total ATP, 4°, 5/10/05						
200 ng/l water prod. point	1304	50	91.05	9	1227	88.38
20 ng/l water prod. point	179	40	10.01	9	102	7.35
2 ng/l water prod. point	94	40	3.89	9	17	1.22
1 ng/l water prod. point	58	30	1.30	9	-19	-1.37
water prod. point	77	30	2.67	9		
200 ng/l milli-Q water	3471	40	233.58	9	3433	233.72
20 ng/ lmilli-Q water	114	30	5.04	9	76	5.17
2 ng/l milli-Q water	50	30	0.68	9	12	0.82
1 ng/l milli-Q water	43	30	0.20	9	5	0.34
milli-Q water	38	30	-0.14	9		

Table 20 continued

	Response RLU	Bkgnd RLU	Concentration ng/l	Degradation time days	Corrected response* RLU	corrected concentration* ng/l
Total ATP, 24°, 4/10/05						
200 ng/l water prod. point	66	50	1.87	10	-13	-0.94
20 ng/l water prod. point	51	40	0.79	10	-28	-2.02
2 ng/l water prod. point	45	30	0.36	10	-34	-2.45
1 ng/l water prod. point	40	30	0.00	10	-39	-2.81
water prod. point	79	40	2.81	10		
200 ng/l milli-Q water	3522	40	237.05	10	3480	236.92
20 ng/l milli-Q water	373	30	22.67	10	331	22.53
2 ng/l milli-Q water	66	30	1.77	10	24	1.63
1 ng/l milli-Q water	56	40	1.09	10	14	0.95
milli-Q water	42	40	0.14	10		
Total ATP, 4°, 4/10/05						
200ng, water prod. point	1446	40	101.27	10	1360	97.96
20 ng/l water prod. point	190	70	10.80	10	104	7.49
2 ng/l water prod. point	92	50	3.75	10	6	0.43
1 ng/l water prod. point	49	30	0.65	10	-37	-2.67
water prod. point	86	30	3.31	10		
200 ng/l milli-Q water	3601	30	242.43	10	3555	242.02
20 ng/l milli-Q water	368	30	22.33	10	322	21.92
2 ng/l milli-Q water	82	40	2.86	10	36	2.45
1 ng/l milli-Q water	67	40	1.84	10	21	1.43
milli-Q water	46	40	0.41	10		

* correction for the ATP content of the water of the water supply or milliQ water

ANNEX 6 INFLUENCE OF STAGNATION TIME ON ATP CONCENTRATION IN TAP WATER SAMPLES

Table 21a Fully Flushed and two successive stagnation samples of 500 ml from Leggiuno on 23 August 2005

Samples	Stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
0(FF)	0	683	60	48.25
0.5HS(1)	0.5	589	30	41.19
0.5HS(2)	0.5	490	40	33.77
0(FF)	0	705	50	49.90
1HS(1)	1	637	50	44.80
1HS(2)	1	533	40	36.99
0(FF)	0	584	50	40.82
2HS(1)	2	541	50	37.59
2HS(2)	2	440	50	30.01
0(FF)	0	525	40	36.39
4HS(1)	4	166	40	9.45
4HS(2)	4	395	40	26.64
0(FF)	0	489	40	33.69
8HS(1)	8	445	50	30.39
8HS(2)	8	431	50	29.34
Free ATP				
FF	0	118	40	3.36
0.5HS(1)	0.5	334	40	12.68
0.5HS(2)	0.5	162	40	5.26
FF	0	172	60	5.69
1HS(1)	1	199	30	6.86
1HS(2)	1	141	40	4.35
FF	0	116	60	3.28
2HS(1)	2	129	70	3.84
2HS(2)	2	226	40	8.02
FF	0	98	40	2.50
4HS(1)	4	166	40	5.43
4HS(2)	4	98	40	2.50
FF	0	97	30	2.46
8HS(1)	8	115	40	3.23
8HS(2)	8	101	40	2.63

Table 21b Fully Flushed and two successive stagnation samples of 500 ml from Laveno on 23 August 2005

Samples	Stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
0(FF)	0	97	50	4.28
0.5HS(1)	0.5	126	60	6.45
0.5HS(2)	0.5	82	70	3.15
0(FF)	0	70	60	2.25
1HS(1)	1	112	50	5.40
1HS(2)	1	104	40	4.80
0(FF)	0	57	40	1.28
2HS(1)	2	129	30	6.68
2HS(2)	2	81	70	3.08
0(FF)	0	64	50	1.80
4HS(1)	4	137	40	7.28
4HS(2)	4	98	40	4.35
0(FF)	0	62	40	1.65
8HS(1)	8	158	50	8.85
8HS(2)	8	73	40	2.48
Free ATP				
FF	0	57	50	0.73
0.5HS(1)	0.5	67	50	1.16
0.5HS(2)	0.5	56	60	0.69
FF	0	49	40	0.39
1HS(1)	1	79	40	1.68
1HS(2)	1	58	40	0.78
FF	0	47	40	0.30
2HS(1)	2	60	40	0.86
2HS(2)	2	63	40	0.99
FF	0	51	40	0.47
4HS(1)	4	69	40	1.25
4HS(2)	4	72	40	1.38
FF	0	53	40	0.56
9.5HS(1)	8	71	40	1.34
9.5HS(2)	8	56	40	0.69

Table 22 Fully Flushed and two successive stagnation samples of 500 ml from Laveno on 20/09/05

Samples	Stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
0(FF)	0	62	40	1.47
0.5HS(1)	0.5	54	20	0.94
0.5HS(2)	0.5	52	40	0.80
0(FF)	0	51	40	0.74
1HS(1)	1	53	40	0.87
1HS(2)	1	57	30	1.14
0(FF)	0	54	40	0.94
2HS(1)	2	71	30	2.07
2HS(2)	2	76	40	2.41
0(FF)	0	49	20	0.60
4HS(1)	4	62	30	1.47
4HS(2)	4	54	40	0.94
0(FF)	0	49	20	0.60
8HS(1)	8	82	30	2.81
8HS(2)	8	70	40	2.01
Free ATP				
0(FF)	0	46	50	0.23
0.5HS(1)	0.5	50	40	0.39
0.5HS(2)	0.5	43	30	0.12
0(FF)	0	49	40	0.35
1HS(1)	1	46	40	0.23
1HS(2)	1	51	30	0.42
0(FF)	0	45	40	0.19
2HS(1)	2	46	40	0.23
2HS(2)	2	50	40	0.39
0(FF)	0	50	30	0.39
4HS(1)	4	45	30	0.19
4HS(2)	4	41	20	0.04
0(FF)	0	46	40	0.23
8HS(1)	8	48	50	0.31
8HS(2)	8	54	50	0.54

Table 23 Fully Flushed and two successive stagnation samples of 500 ml from Leggiuno on 20/09/05

Samples	Stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
0(FF)	0	409	10	24.69
0.5HS(1)	0.5	366	40	21.81
0.5HS(2)	0.5	256	40	14.45
0(FF)	0	439	40	26.69
1HS(1)	1	374	30	22.35
1HS(2)	1	305	40	17.73
0(FF)	0	824	40	52.45
2HS(1)	2	480	40	29.44
2HS(2)	2	331	40	19.47
0(FF)	0	272	60	15.52
4HS(1)	4	242	40	13.51
4HS(2)	4	229	30	12.64
0(FF)	0	271	30	15.45
8HS(1)	8	251	50	14.12
8HS(2)	8	248	50	13.92
Free ATP				
0(FF)	0	125	40	3.27
0.5HS(1)	0.5	159	60	4.58
0.5HS(2)	0.5	187	40	5.66
0(FF)	0	110	40	2.70
1HS(1)	1	105	30	2.50
1HS(2)	1	87	40	1.81
0(FF)	0	104	30	2.47
2HS(1)	2	151	30	4.28
2HS(2)	2	91	30	1.96
0(FF)	0	828	40	30.36
4HS(1)	4	102	40	2.39
4HS(2)	4	182	30	5.47
0(FF)	0	273	40	8.98
8HS(1)	8	102	40	2.39
8HS(2)	8	118	30	3.00

Table 24 ATP concentration in 20 successive tap water samples of 50 ml from Leggiuno after 8 h stagnation on 16/08/05

Samples	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP			
FF	371	40	21.93
1	214	60	11.53
2	185	50	9.61
3	338	40	19.74
4	274	50	15.50
5	310	40	17.89
6	287	50	16.36
7	268	40	15.10
8	339	40	19.81
9	314	50	18.15
10	312	70	18.02
11	306	40	17.62
12	334	40	19.48
13	279	60	15.83
14	307	70	17.69
15	292	40	16.69
16	312	40	18.02
17	383	70	22.72
18	302	70	17.36
19	297	40	17.03
20	340	40	19.87
Free ATP			
FF	112	60	2.83
1	118	40	3.07
2	86	30	1.81
3	106	50	2.59
4	82	50	1.65
5	93	40	2.08
6	122	50	3.22
7	124	40	3.30
8	138	40	3.85
9	146	70	4.17
10	146	40	4.17
11	209	60	6.64
12	110	50	2.75
13	93	40	2.08
14	90	40	1.97
15	110	50	2.75
16	118	50	3.07
17	82	60	1.65
18	97	40	2.24
19	85	60	1.77
20	102	50	2.44

Table 25 ATP concentration in 20 successive tap water samples of 50 ml from Laveno after 8 h stagnation on 16/08/05

Samples	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP			
FF	148	60	7.15
1	249	50	13.85
2	272	50	15.37
3	362	60	21.33
4	158	40	7.82
5	172	50	8.74
6	129	40	5.90
7	101	20	4.04
8	89	40	3.25
9	225	40	12.26
10	104	70	4.24
11	101	60	4.04
12	82	60	2.78
13	80	50	2.65
14	87	150	3.11
15	80	70	2.65
16	76	60	2.38
17	68	50	1.85
18	69	40	1.92
19	104	40	4.24
20	71	40	2.05
Free ATP			
FF	99	60	2.32
1	69	40	1.14
2	134	40	3.69
3	98	40	2.28
4	101	40	2.40
5	90	30	1.97
6	79	40	1.53
7	71	40	1.22
8	70	50	1.18
9	62	50	0.86
10	58	30	0.71
11	71	50	1.22
12	59	40	0.75
13	60	40	0.79
14	60	80	0.79
15	54	30	0.55
16	71	40	1.22
17	56	50	0.63
18	65	40	0.98
19	96	40	2.20
20	54	40	0.55

Table 26 ATP concentrations in samples from the DTF data on 01/07/05

Samples polypropene	Stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
line15 p.1	8	145	50	5.89
line15 p.2	0.5	306	90	13.40
line15 p.3	1	262	30	14.39
line15 p.4	2	202	40	10.05
line15 p.5	0.5	206	50	9.68
line15 p.6	4	165	40	7.75
line15 p.7	1	163	40	7.63
line15 p.8	16	168	40	7.94
water prod. Point		352	30	
Free ATP				
line 15 p.1	8	180	20	6.91
line 15 p.2	0.5	409	40	15.93
line 15 p.3	1	358	50	13.29
line 15 p.4	2	262	40	9.58
line 15 p.5	0.5	297	40	11.09
line 15 p.6	4	225	40	7.98
line 15 p.7	1	202	40	6.99
line 15 p.8	16	139	40	4.27
water prod. Point		473	40	

Samples Gavanised steel	Stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
line14 p.1	8	123	50	4.53
line14 p.2	0.5	237	60	10.98
line14 p.3	1	198	40	9.80
line14 p.4	2	127	40	5.40
line14 p.5	0.5	176	40	8.43
line14 p.6	4	79	60	1.18
line14 p.7	1	118	50	4.22
line14 p.8	16	75	30	2.79
free ATP				
line14 p.1	8	157	40	5.05
line14 p.2	0.5	313	30	12.21
line14 p.3	1	262	40	9.58
line14 p.4	2	139	40	4.27
line14 p.5	0.5	232	50	7.86
line14 p.6	4	90	60	1.29
line14 p.7	1	151	40	4.79
line14 p.8	16	66	50	0.69

Table 26 continued

Samples	Stagnation time	Response	Bkgnd	Concentration
Stainless steel	h	RLU	RLU	ng/l
Total ATP				
line13 p.1	8	151	60	5.64
line13 p.2	0.5	286	40	15.26
line13 p.3	1	267	60	12.84
line13 p.4	2	218	40	11.04
line13 p.5	0.5	221	40	11.23
line13 p.6	4	182	40	8.81
line13 p.7	1	190	60	8.06
line13 p.8	16	159	30	8.00
free ATP				
line13 p.1	8	185	40	6.26
line13 p.2	0.5	395	30	15.75
line13 p.3	1	374	50	13.98
line13 p.4	2	295	40	11.01
line13 p.5	0.5	286	40	10.62
line13 p.6	4	251	40	9.11
line13 p.7	1	221	40	7.81
line13 p.8	16	188	50	5.96

Samples	Stagnation time	Response	Bkgnd	Concentration
Copper	h	RLU	RLU	ng/l
Total ATP				
line 12 p.1	8	173	40	8.25
line 12 p.2	0.5	315	70	15.20
line 12 p.3	1	283	50	14.45
line 12 p.4	2	211	70	8.75
line 12 p.5	0.5	235	40	12.09
line 12 p.6	4	146	60	5.33
line 12 p.7	1	158	60	6.08
line 12 p.8	16	71	40	1.92
Free ATP				
line12 p.1	8	232	40	8.29
line12 p.2	0.5	414	50	15.71
line12 p.3	1	393	40	15.24
line12 p.4	2	277	30	10.66
line12 p.5	0.5	314	40	11.83
line12 p.6	4	182	70	4.83
line12 p.7	1	193	40	6.60
line12 p.8	16	59	40	0.82

Table 27 ATP concentrations in samples from the DTF data on 20/09/05

Samples Copper		stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP					
	12.1	8	65	40	1.67
	12.2	0.5	113	30	4.88
	12.3	1	73	50	2.21
	12.4	2	104	40	4.28
	12.5	0.5	126	40	5.75
	12.6	4	95	40	3.68
	12.7	1	114	40	4.95
	12.8	16	51	40	0.74
Free ATP					
	12.1	8	132	40	3.54
	12.2	0.5	325	60	10.98
	12.3	1	298	50	9.94
	12.4	2	241	40	7.74
	12.5	0.5	252	30	8.17
	12.6	4	176	70	5.24
	12.7	1	221	30	6.97
	12.8	16	71	30	1.19

Samples Stainless steel		stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP					
	13.1	8	280	40	16.06
	13.2	0.5	386	30	23.15
	13.3	1	382	40	22.88
	13.4	2	321	30	18.80
	13.5	0.5	356	40	21.14
	13.6	4	275	40	15.72
	13.7	1	262	40	14.85
	13.8	16	216	30	11.78
Free ATP					
	13.1	8	151	30	4.28
	13.2	0.5	183	20	5.51
	13.3	1	165	40	4.82
	13.4	2	169	40	4.97
	13.5	0.5	178	30	5.32
	13.6	4	154	40	4.39
	13.7	1	151	70	4.28
	13.8	16	151	40	4.28

Table 27 continued

Samples Galvanised steel	stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
14.1	8	156	20	7.76
14.2	0.5	108	30	4.55
14.3	1	112	50	4.82
14.4	2	138	40	6.56
14.5	0.5	109	40	4.62
14.6	4	121	40	5.42
14.7	1	101	40	4.08
14.8	16	319	40	18.67
Free ATP				
14.1	8	48	40	0.31
14.2	0.5	103	40	2.43
14.3	1	90	40	1.93
14.4	2	68	20	1.08
14.5	0.5	108	60	2.62
14.6	4	56	40	0.62
14.7	1	79	40	1.50
14.8	16	67	40	1.04

Samples polypropene	stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
Total ATP				
15.1	8	85	40	3.01
15.2	0.5	102	40	4.15
15.3	1	107	40	4.48
15.4	2	102	30	4.15
15.5	0.5	110	20	4.68
15.6	4	92	50	3.48
15.7	1	110	40	4.68
15.8	16	102	40	4.15
FF DTF	0	96	40	3.75
Free ATP				
15.1	8	94	20	2.08
15.2	0.5	110	50	2.70
15.3	1	110	40	2.70
15.4	2	110	30	2.70
15.5	0.5	119	40	3.04
15.6	4	109	40	2.66
15.7	1	109	40	2.66
15.8	16	84	40	1.70
FF DTF	0	115	30	2.89

Table 28 Overview of ATP concentrations in tap water samples from different distribution systems on 23/08/05

Samples	Stagnation time h	Response RLU	Bkgnd RLU	Concentration ng/l
total ATP				
Laveno	FF	68	50	2.10
Laveno	09:30	104	60	4.80
Laveno	09:30	71	40	2.33
Foresteria	FF	157	40	8.78
Foresteria	07:15	110	50	5.25
Foresteria	07:15	119	70	5.93
sterile water		42	40	0.15
water prod. point		154	40	8.55
Varese	FF	60	70	1.50
Varese	04:40	85	60	3.38
Varese	04:40	82	50	3.15
Ranco1	FF	71	70	2.33
Ranco1	07:50	123	60	6.23
Ranco1	07:50	72	60	2.40
Cadrezzate	FF	68	40	2.10
Cadrezzate	07:50	93	60	3.98
Cadrezzate	07:50	64	40	1.80
sterile water		48	60	0.60
water prod. point		144	50	7.80
Ranco2	FF	50	40	0.75
Ranco2	08:30	126	40	6.45
Ranco2	08:30	54	40	1.05
free ATP				
Laveno	FF	53	60	0.56
Laveno	09:30	60	50	0.86
Laveno	09:30	62	30	0.95
Foresteria	FF	74	40	1.47
Foresteria	07:15	85	40	1.94
Foresteria	07:15	71	50	1.34
sterile water		49	40	0.39
water prod. point		138	40	4.23
Varese	FF	61	40	0.91
Varese	04:40	68	40	1.21
Varese	04:40	93	40	2.29
Ranco1	FF	58	40	0.78
Ranco1	07:50	71	40	1.34
Ranco1	07:50	65	60	1.08
Cadrezzate	FF	66	40	1.12
Cadrezzate	07:50	51	60	0.47
Cadrezzate	07:50	62	50	0.95
sterile water		40	40	0.00
water prod.point		135	40	4.10
Ranco2	FF	51	40	0.47
Ranco2	08:30	76	40	1.55
Ranco2	08:30	53	40	0.56

ANNEX 7 HETEROTROPHIC PLATE COUNTS OF TAP WATER SAMPLES

Table 29 HPC of samples taken on 24/05/05

Samples	PCA 22°C		PCA 37°C		R2A 22°C		R2A 37°C	
	cfu/ml	Note	cfu/ml	Note	cfu/ml	Note	cfu/ml	Note
control plate	0		0		0		0	
001	0		0		1	a, b	0	
002	3	a, b	0		211	a, b	112	a, b
003	2	a, b	0		194	a, b	108	a, b
004	0		1	a, b	200	a, b	108	g
005	2	a, b	0		2	a, b	1	a, c
006	0		0		2	a, b	3	a, b
007	1	a, b	1	a, b	1	a, b	0	g
008	1	a, b	0		2	i	3	a, b
009	1	i	0	f	0		1	a, b
010	1	a, b	1	g	20	a, b	21	b
011	0	f	0	f	27	a, b	177	b, d
012	0	f	10	a, b	44	a, b	251	b, c (20*), d
013	0	f	11	c (1*), h	1600	a, b, e	41	a, b
014	0	f	4	f	40	a, b	622	f

a small colonies
b white colonies
c yellow colonies
d 1 colony 0.5 cm Ø
e circa 400 colonies in one quarter
f medium not transparent
g medium was 33-50% dry
h solid particles
i near the number of counted colonies means that the calculation was very tentative, due to fact that it was difficult to decide whether there have been colonies or solid particles

Table 30 HPC of samples taken on 01/06/05

Samples	PCA 22°C		PCA 37°C		R2A 22°C		R2A 37°C	
	cfu/ml	Note	cfu/ml	Note	cfu/ml	Note	cfu/ml	Note
control plate	0		0		0		0	
001	400	a,b,c	720	a,b,c	1200	a,b,c	900	a,b,c
002	249	a,b,c	318	a,b,c	900	a,b,c	550	a,c,f
003	25	a,b	18	a,b,c,d(1*)	74	a,b,c	32	a,c,f
004	4000	a,b	4000	a,b,d(2*)	4000	a,b,c	4000	a,c,d(3*)
005	45	a,b,d	106	a,b,c(1*;5mm Ø)	250	a,b,c	100	a,c,d(1*),f
006	184	a,b	200	a,c,d(1*)	600	a,b,c,e	450	a,c(1*),d(1*)
007	500	a,c	500	a,c	700	a,c	450	a,c
008	59	a,b	26	a,b	181	a,b	81	a,f
009	3	a,b	2	a,b,d	0		5	a,b
010	44	a,b	1	a,b,d	700	a,b,c,f	123	a,b,c,f
011	79	a,b	5	a,c,d	1100	a,b,c,f	202	a,b,c,f
012	107	a,b	4	a,b,d	800	a,c	183	a,b,c,f
013	0		2	a,b	0		1	d

a small colonies
b white colonies
c yellow colonies
d fungi
d medium is opaque
e brown colonies
f orange colonies

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